

# HLA-A PURIFICATION AND PEPTIDE ELUTION

Cellular Source: HLA-Antigens  
( $5\text{-}10 \times 10^6$  cell equivalents)

- a) EBV transformed B cell lines - homozygous
- b) HLA-A transfectants - e.g. .221-HLA-A1
- c) P815 transfectants (mouse mastocytoma)

Detergent Lysis  
( $10^6$  cells/ml)

1% NP-40 or 1% Renex 30 plus protease inhibitors - 1 hr, 4°C

Detergent Lysate

Centrifugation at 15,000xg, 30 min.

Affinity Chromatography

mAb-Sepharose 5 mg/ml  
5-10 ml column

Purified HLA-A Antigen

Anticipated yields 450-800 µg

Acid Treatment

10% acetic acid, 5 min, 100°C

Peptides

YM3 filtration, 3kD cut-off

Sequence/Motif

D. Hunt - HPLC/EI-TMS  
Cytel - HPLC/ABI 477A

09665540 - 0974300

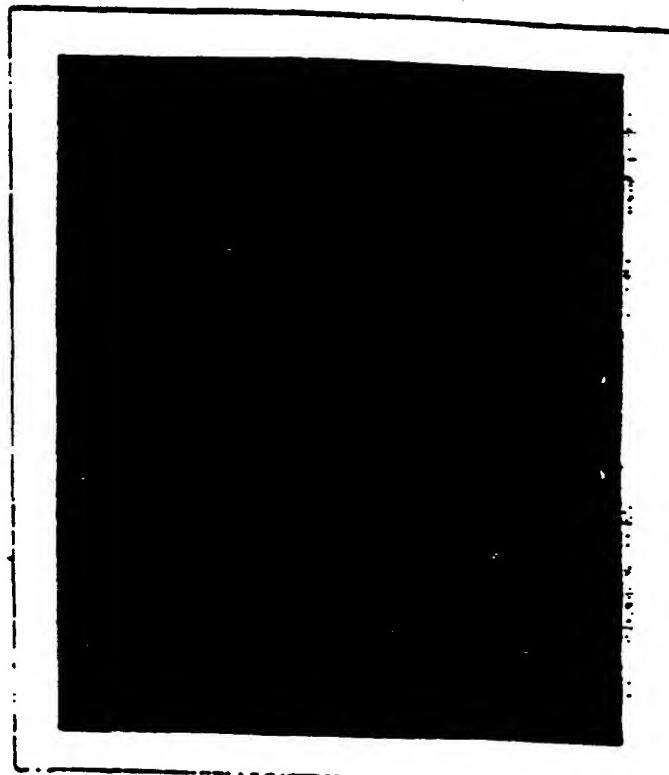
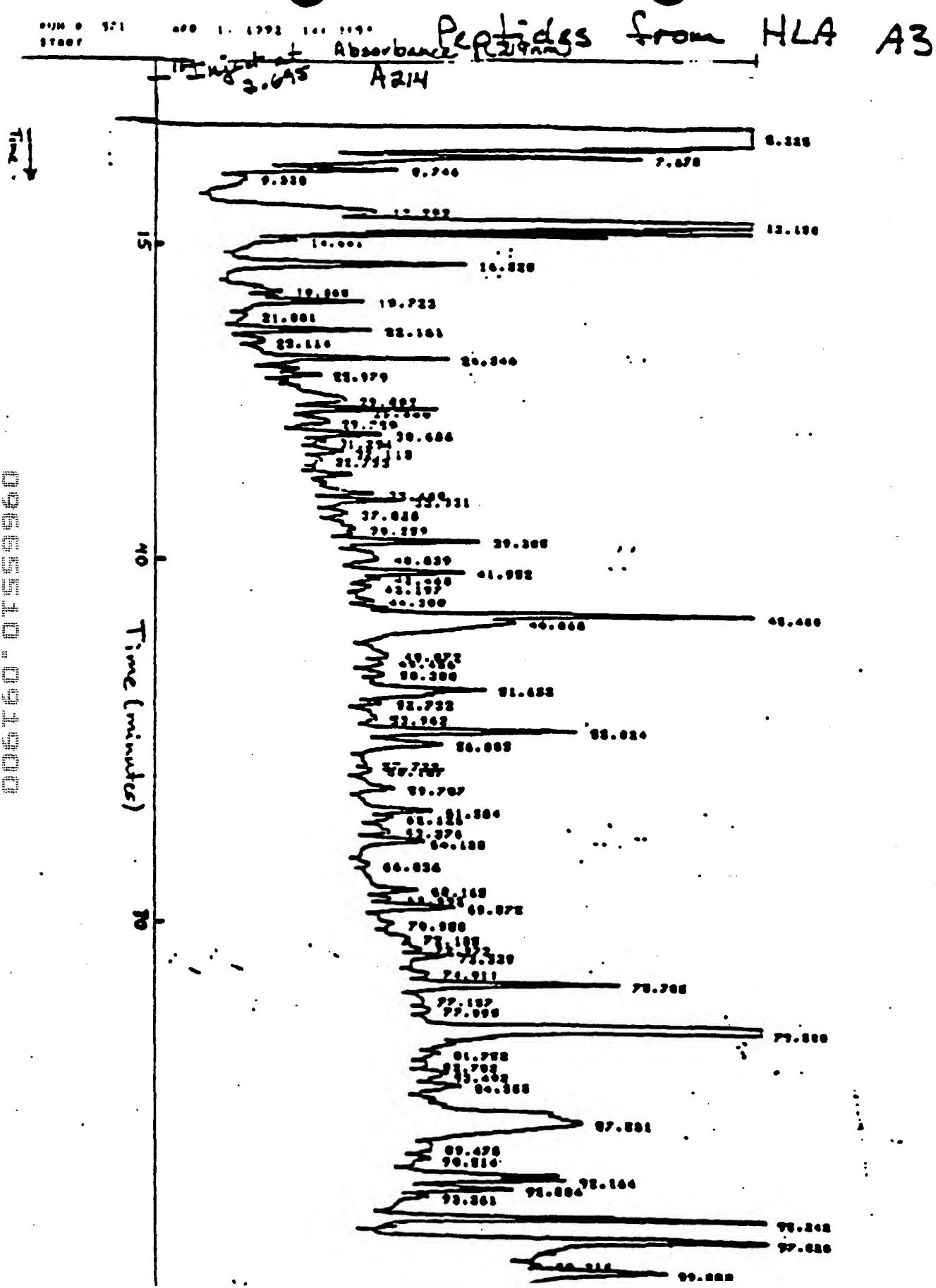


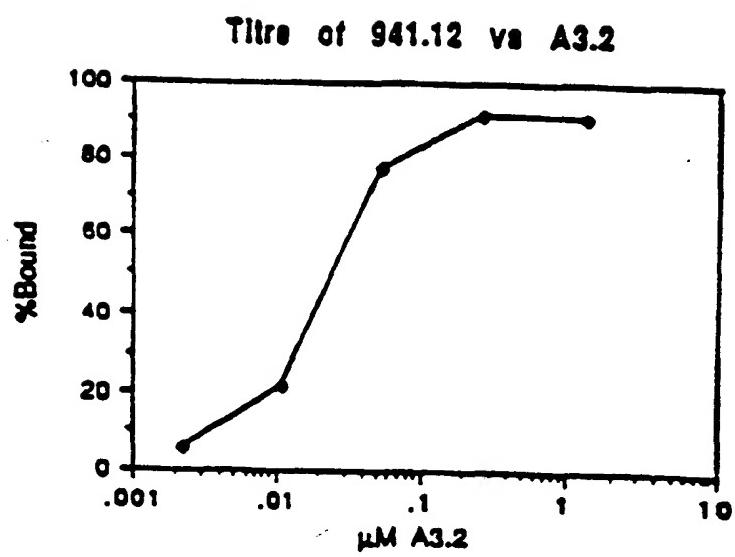
Figure 2

RP-HPLC separation of HLA-A3 acid eluted peptides.



O 96 E 4.0 - O 94 4.260

941.12 vs A3.2



00065T00 - 00100

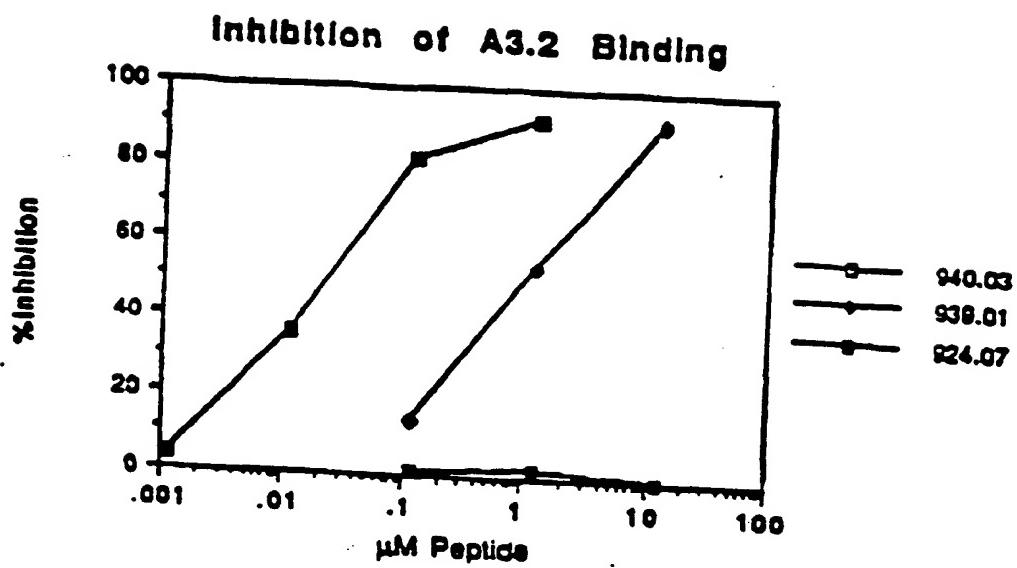


Figure 5

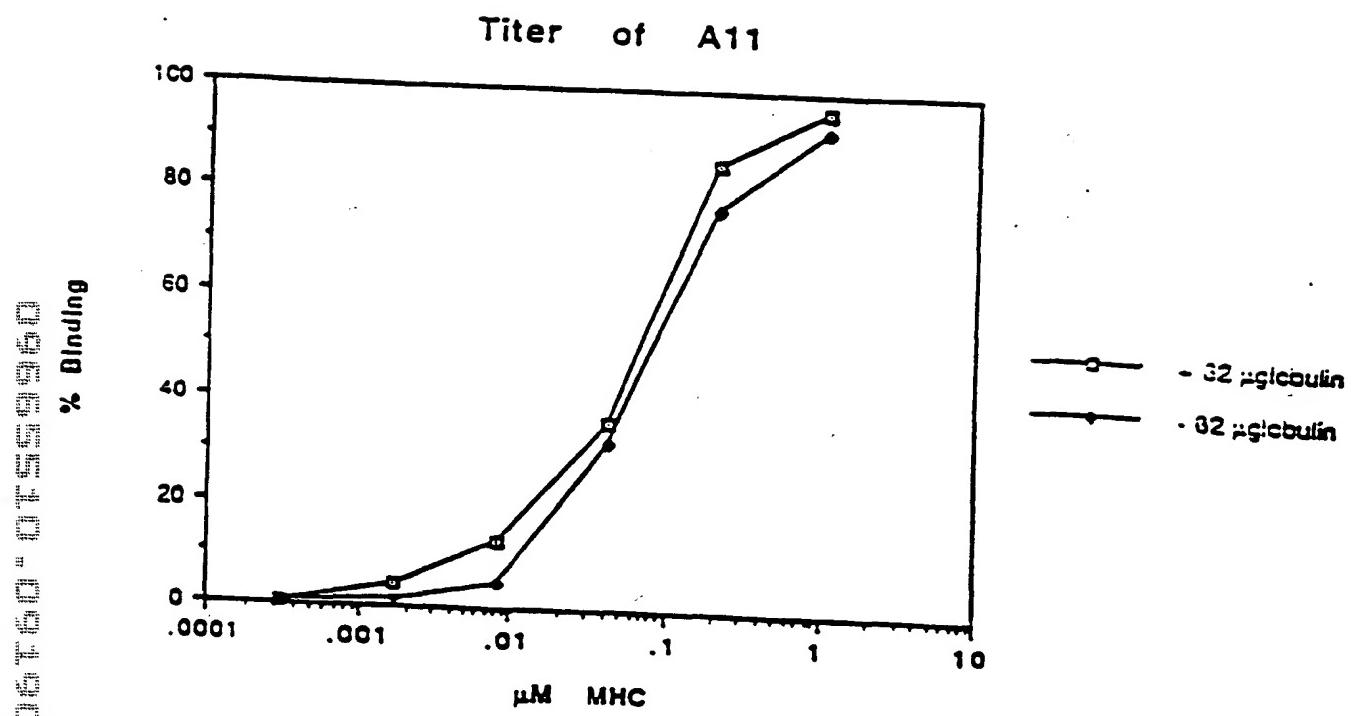


Figure 6

Percent Inhibition

Titer of 940.06

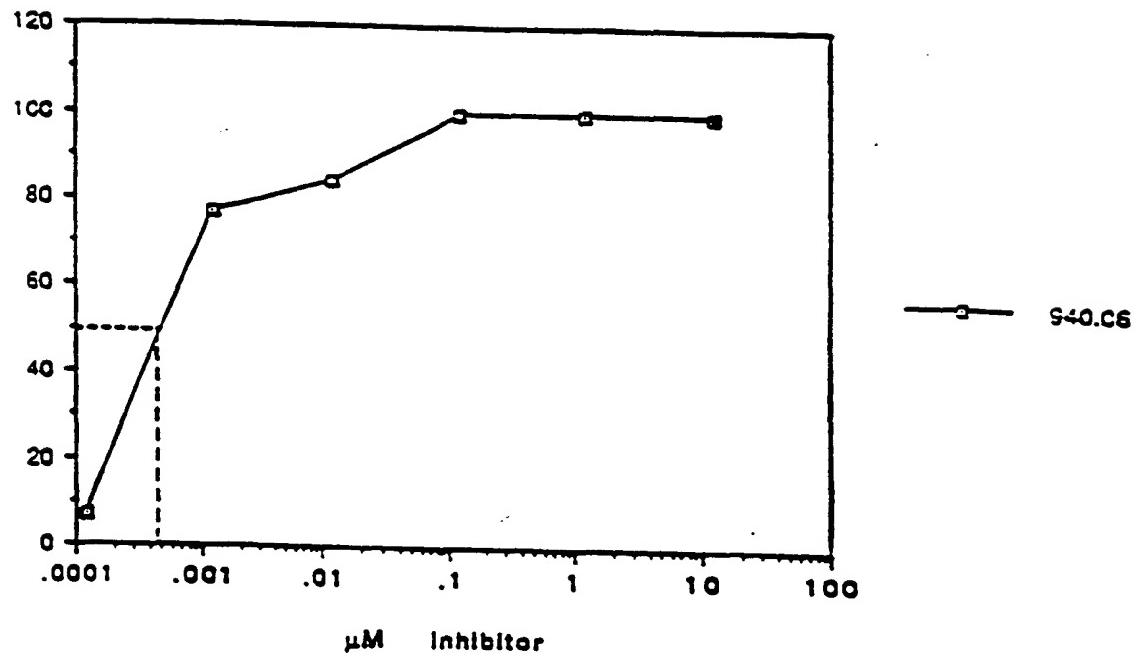


Figure 7

00064760 - 011332960

A11 Scatchard

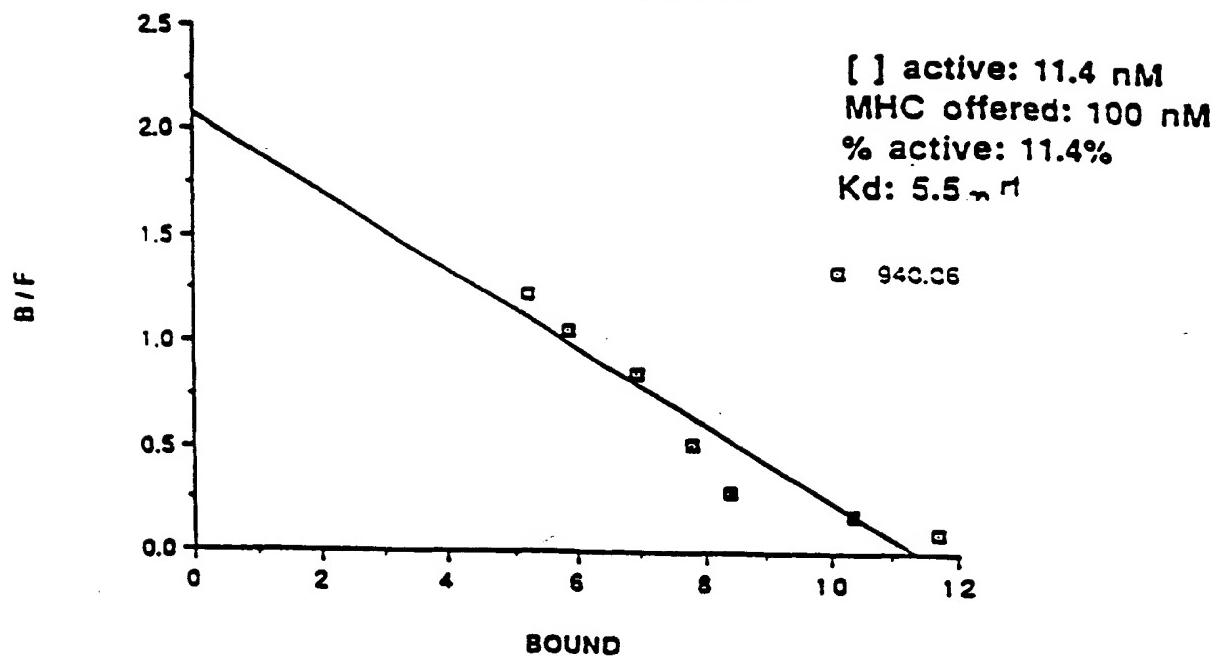


Figure 8

Titer of 944.02 against Al.

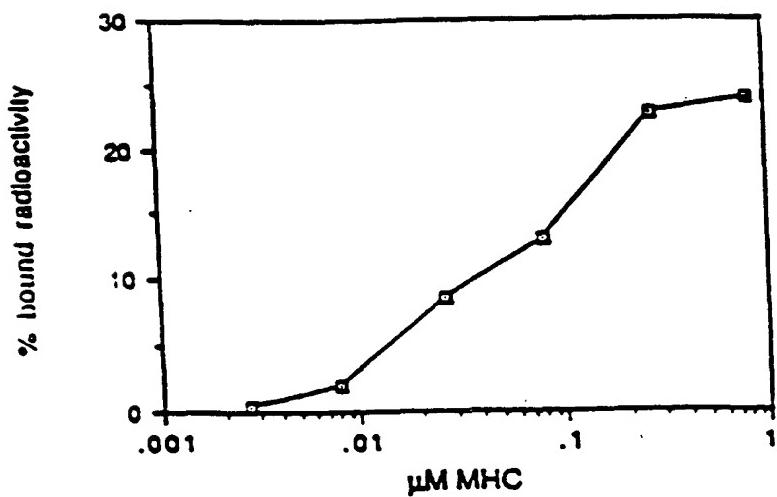


Figure 9

3X

A1 inhibition titration.

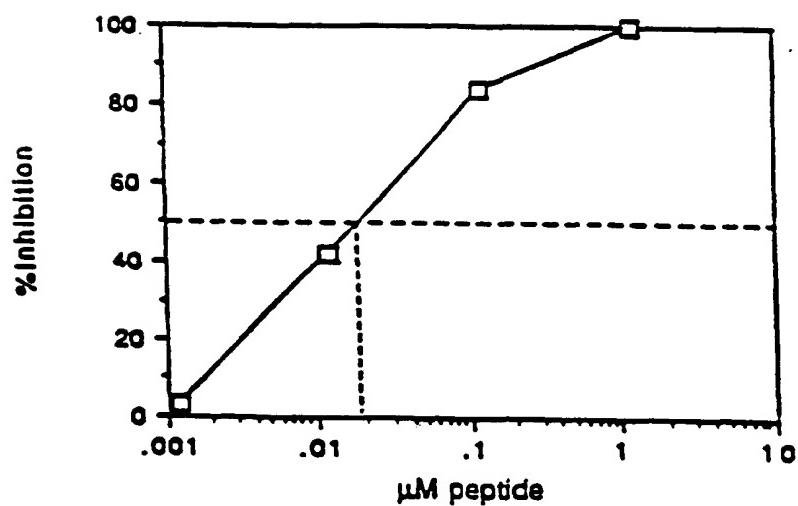


Figure 10

096634C 094300

Scatchard for A1 vs. 944.02

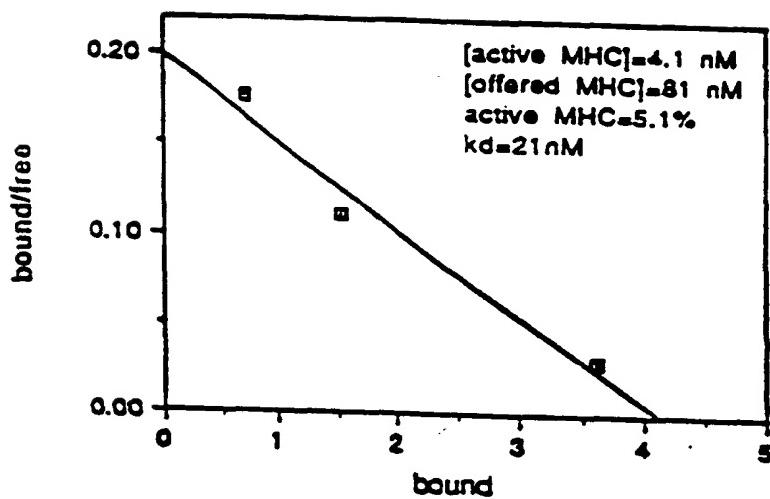


Figure 11

O O E E E E E E E E

A24 titration.

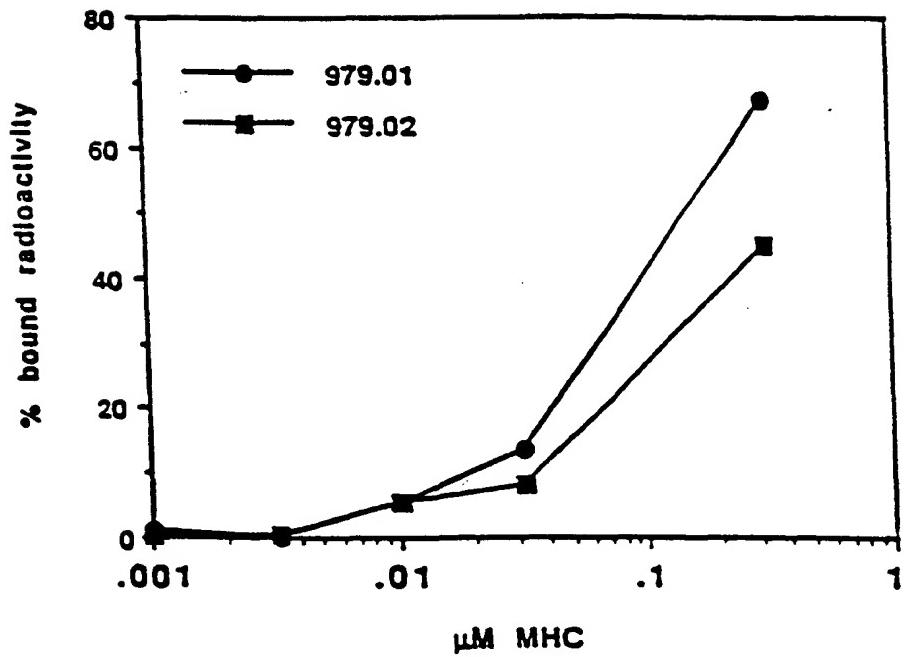


Figure 12

906750-01559360

A24 Inhibition.

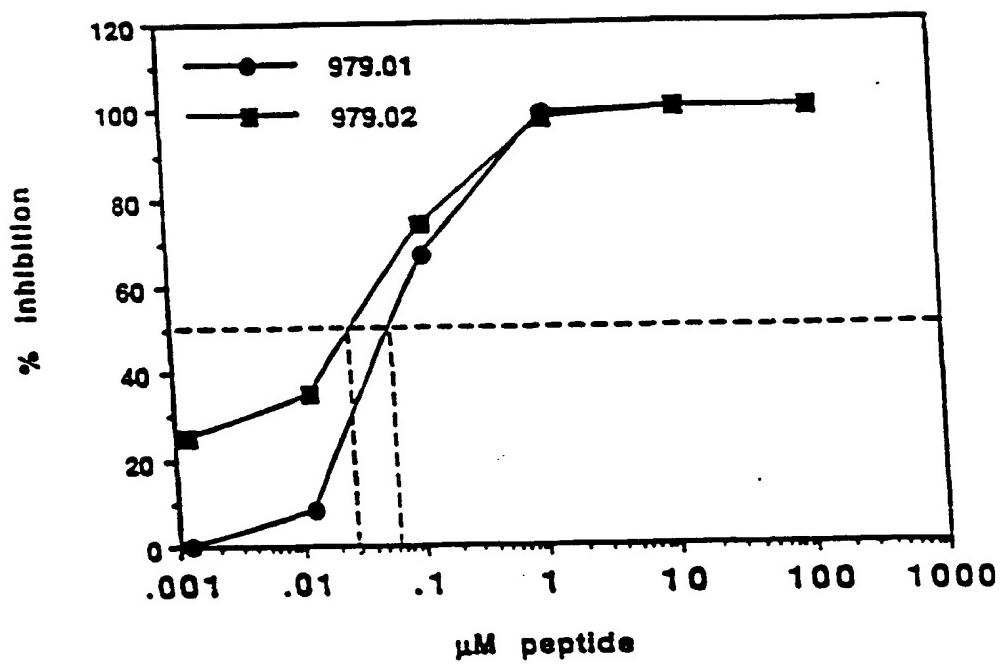


Figure 13

0006160 - 0115533660

Figure 14A

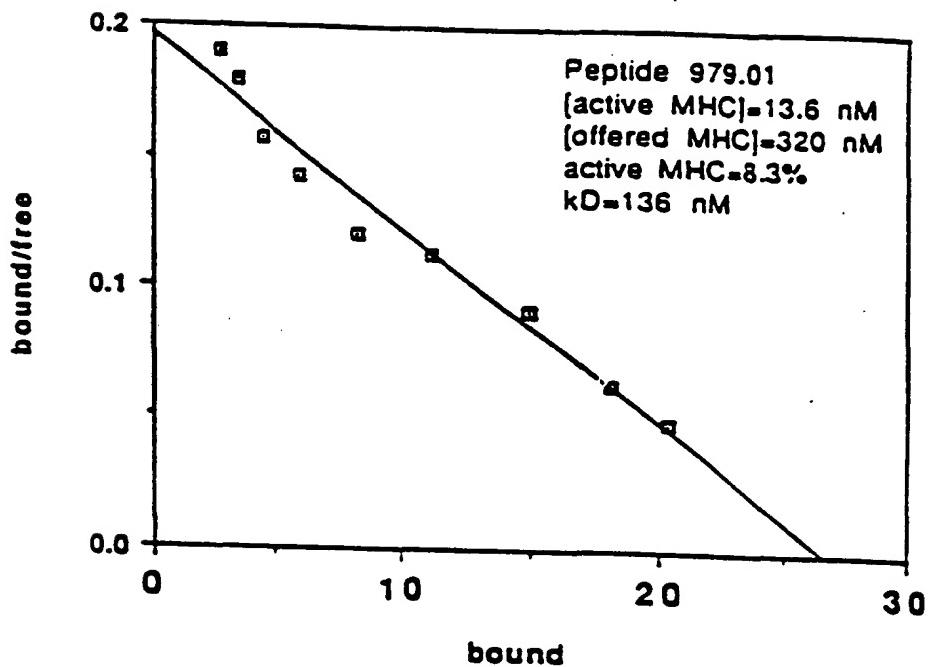


Figure 14B

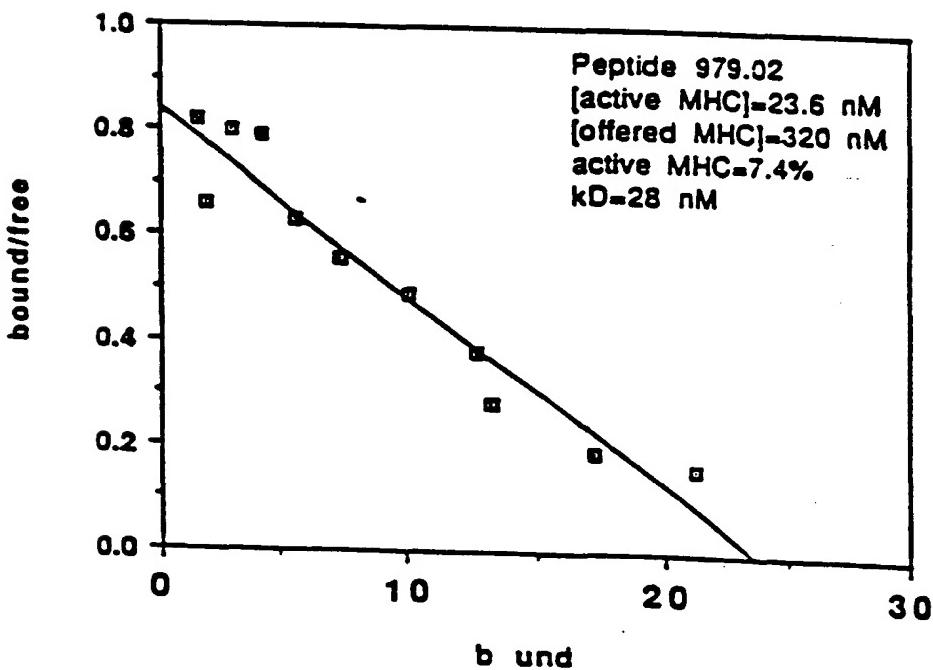
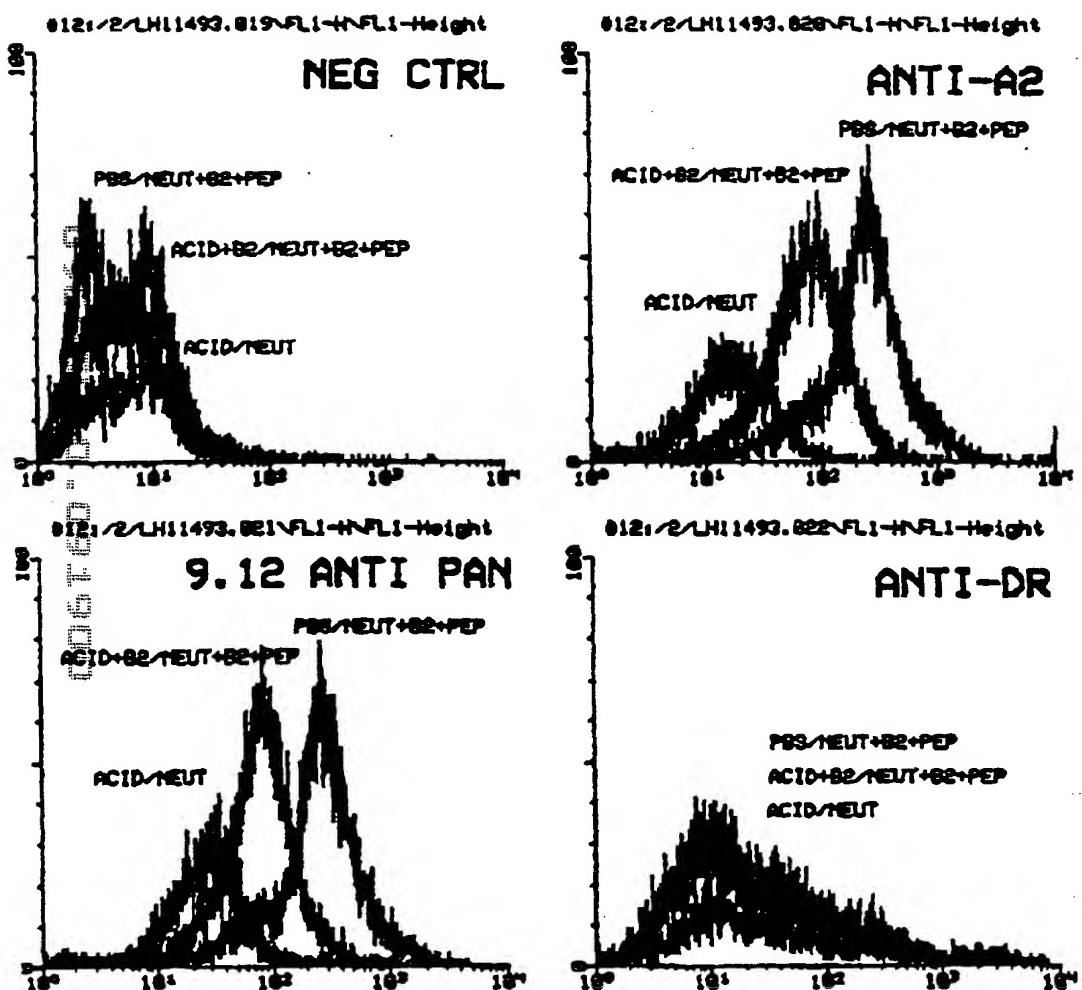


Figure 15



00057600 DT6593610

Figure 16

**CTL INDUCTION**  
**GC43 A2.1 CD4 DEPLETED RESPONDER**  
**777.03 + 924.07 + 927.32 POOL**

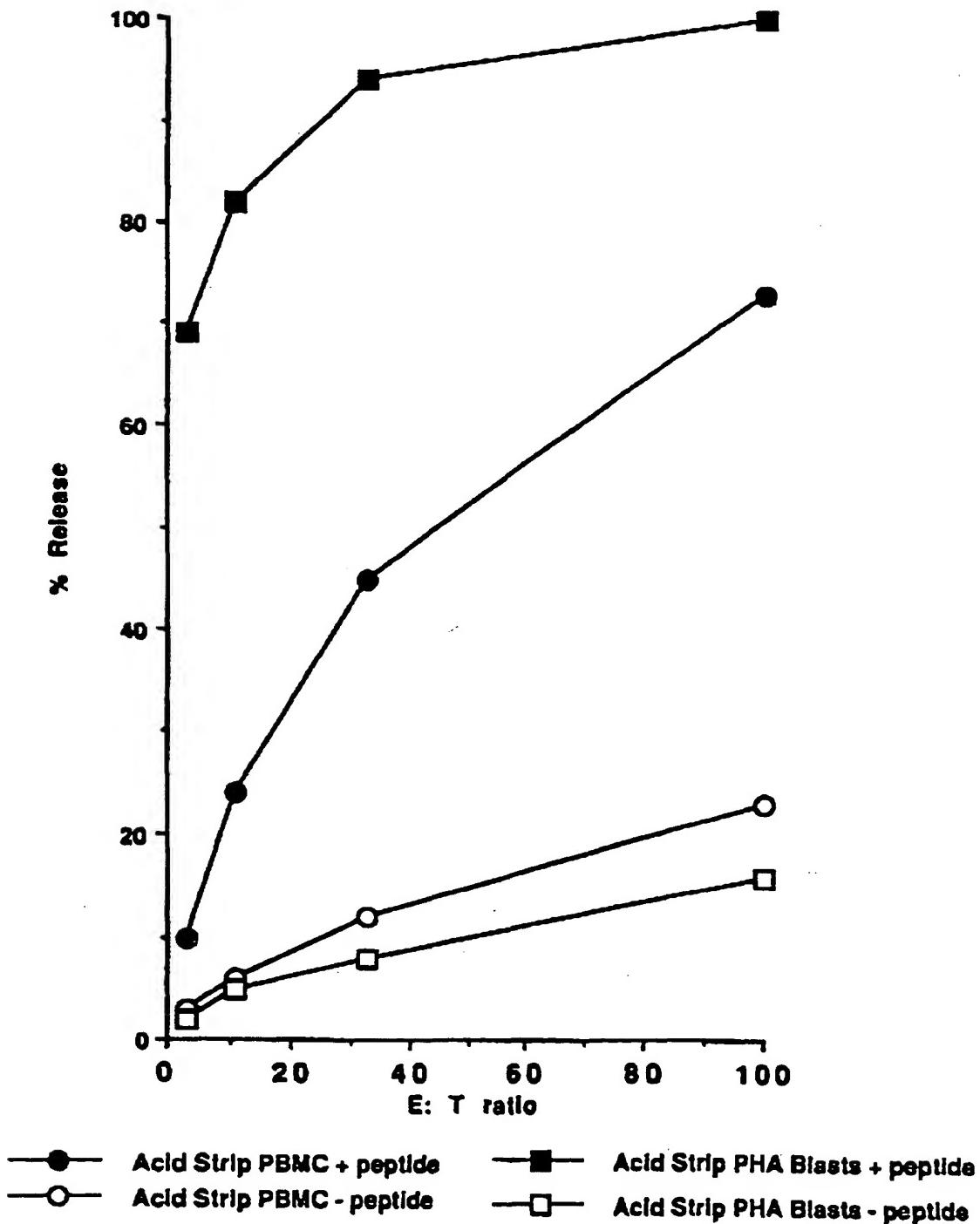
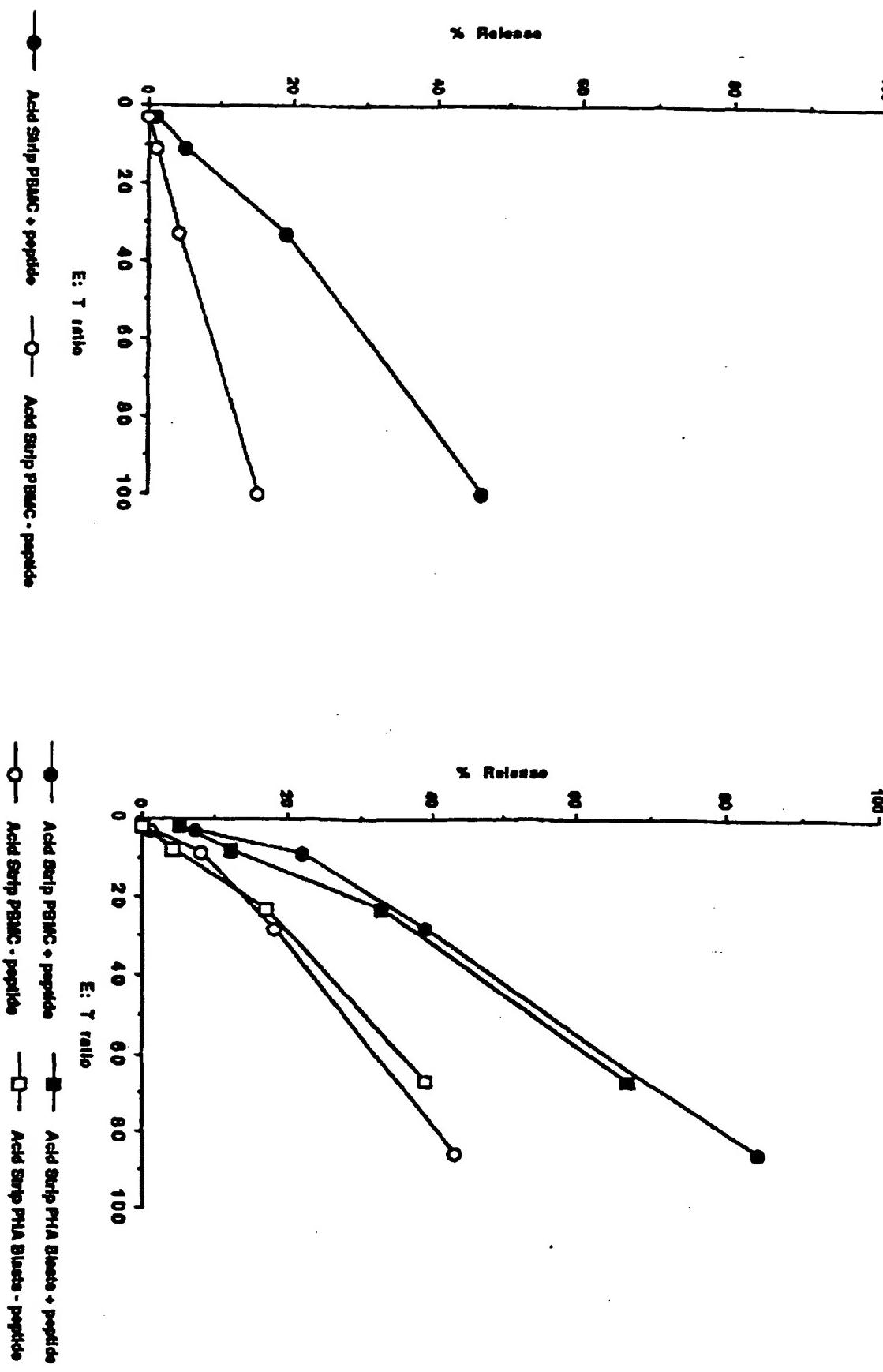


Figure 1/



0 20 40 60 80 100 - C G T A G C G C G

Figure 18

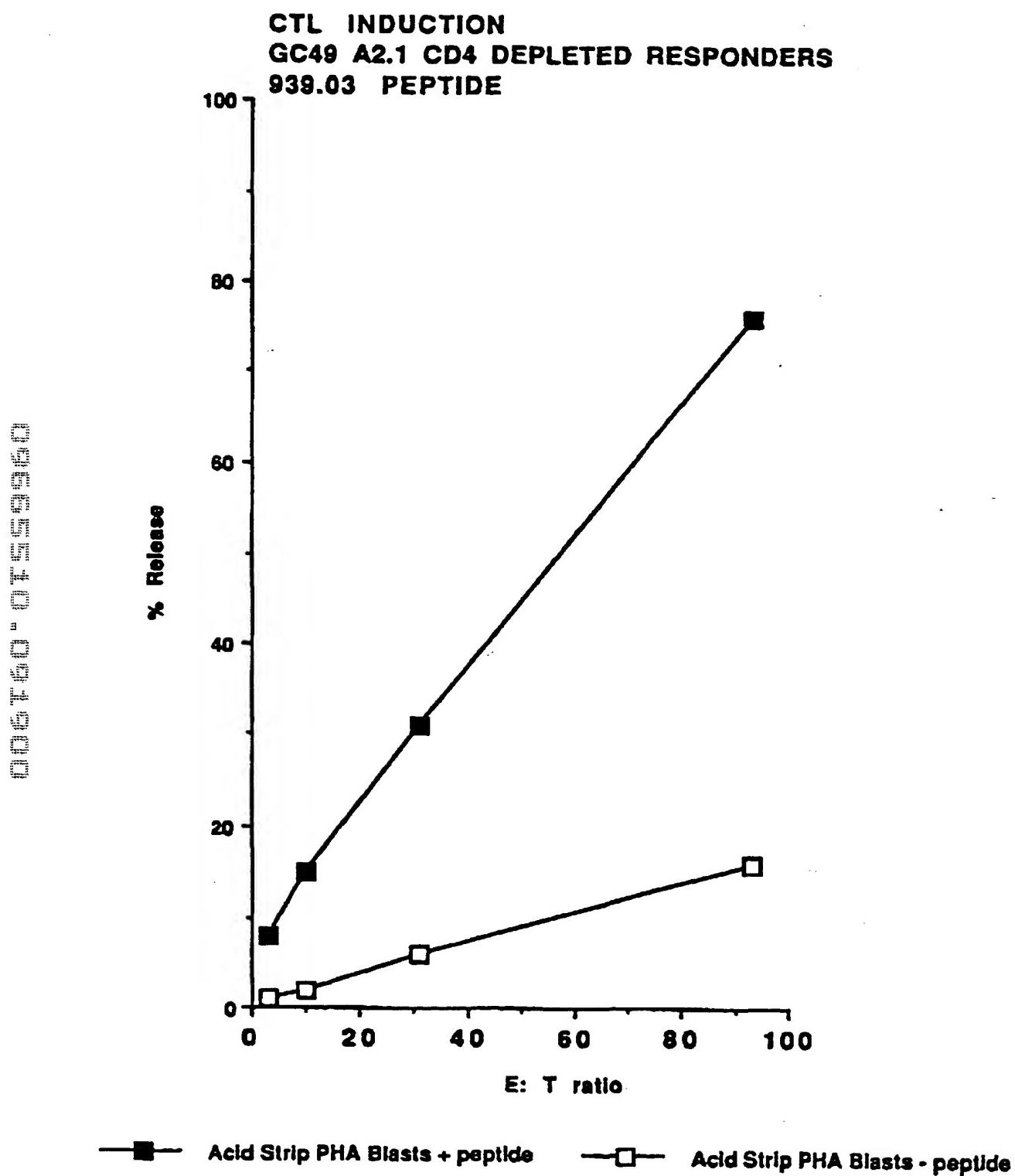


Figure 19

MAGE1 Peptide Specific, HLA-A1-Restricted CTL Can Kill Melanoma Cells Expressing the Endogenous Antigen

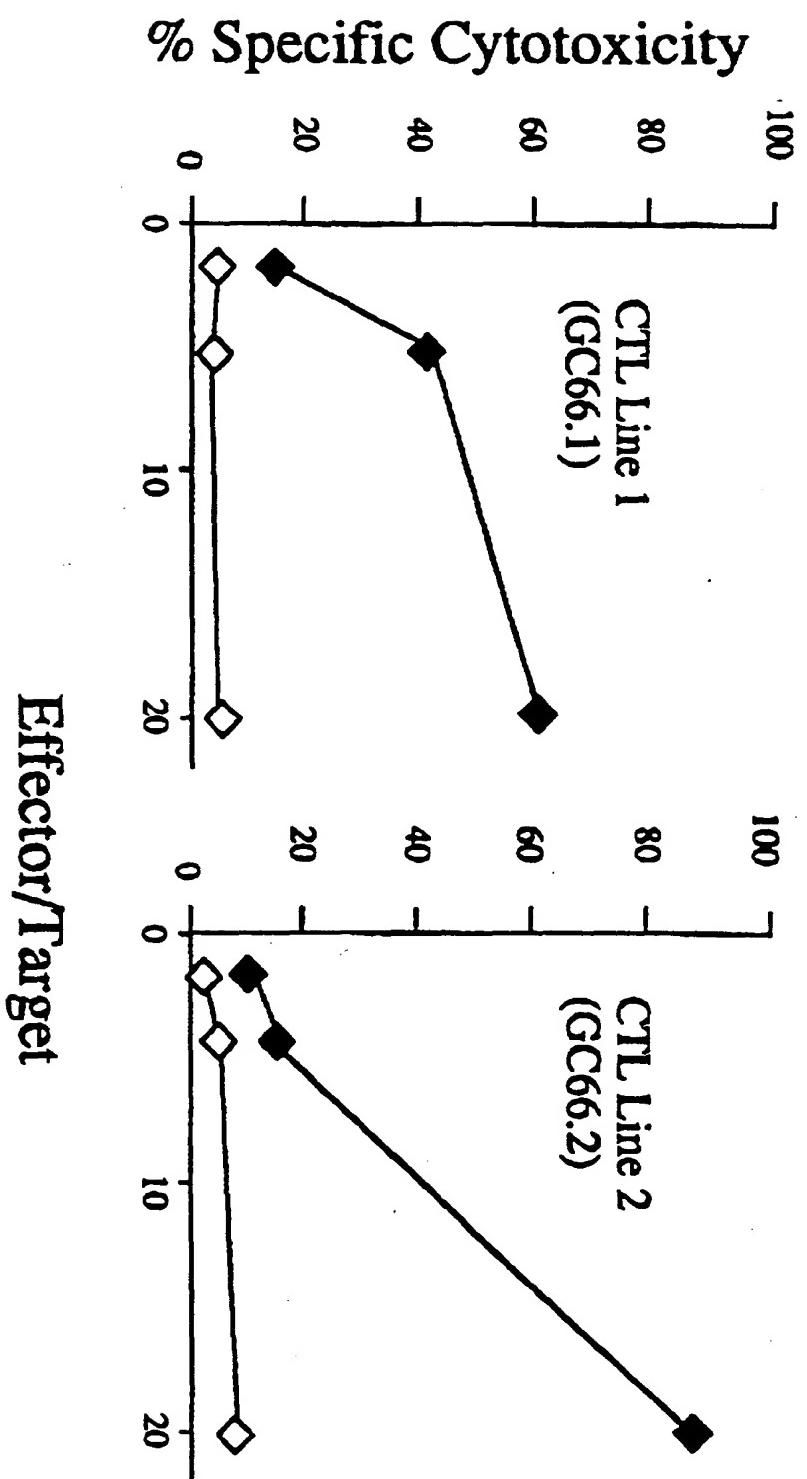
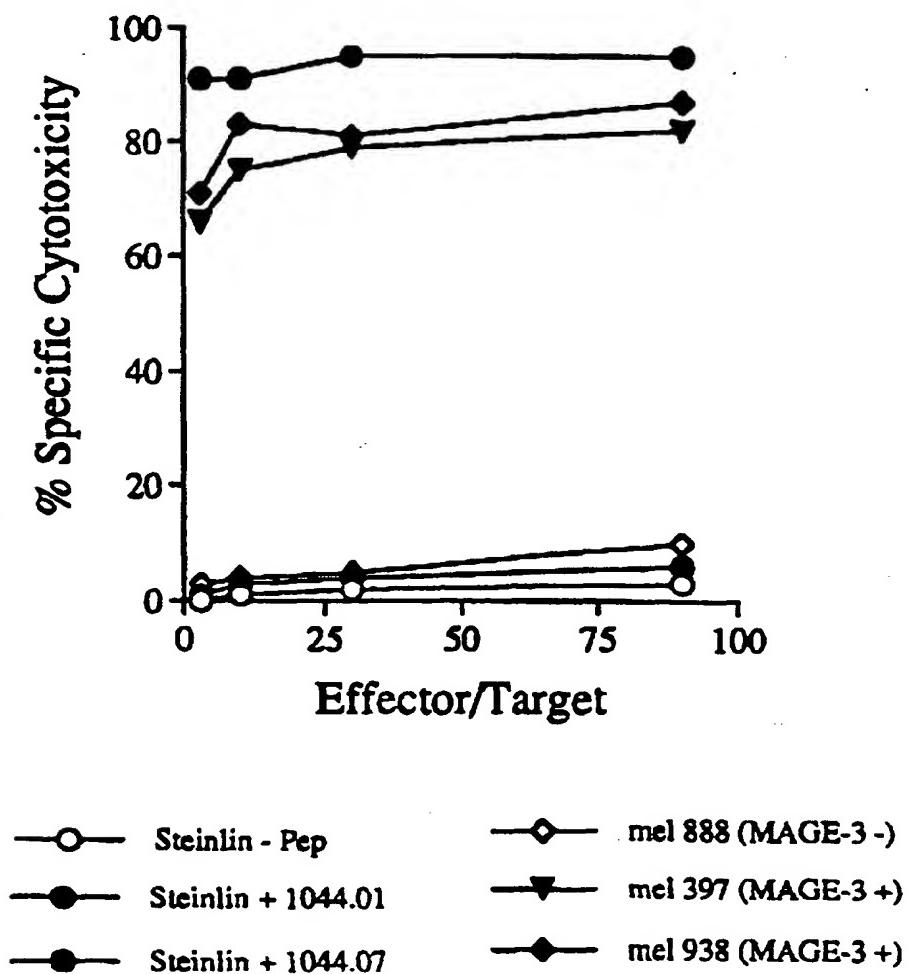


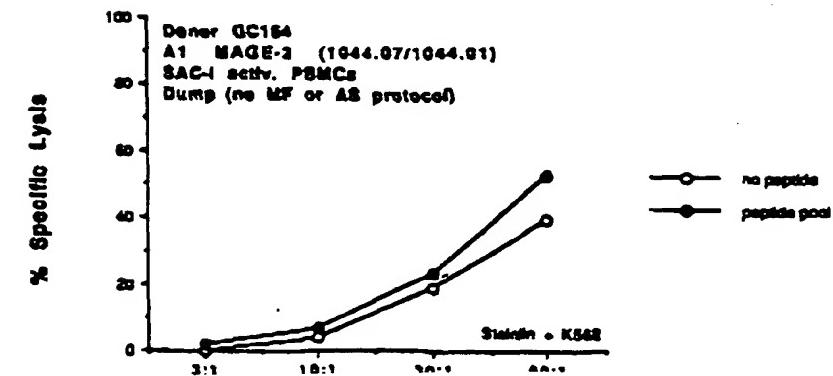
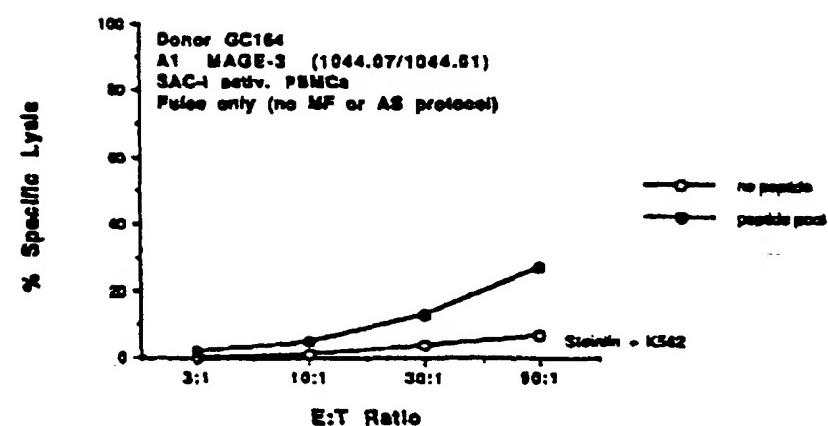
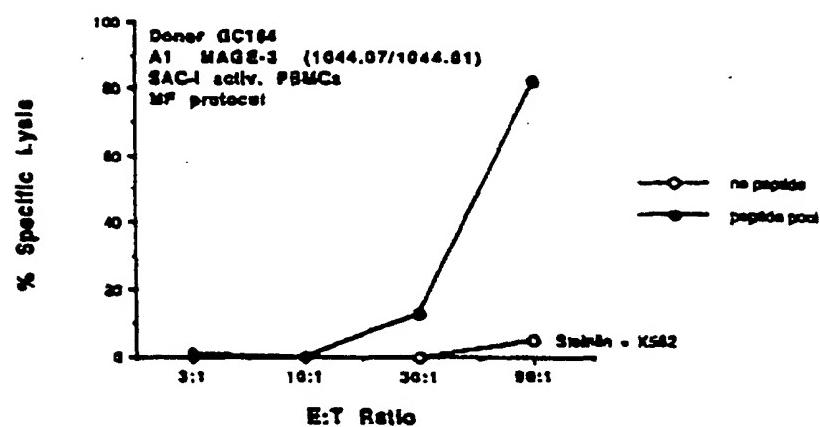
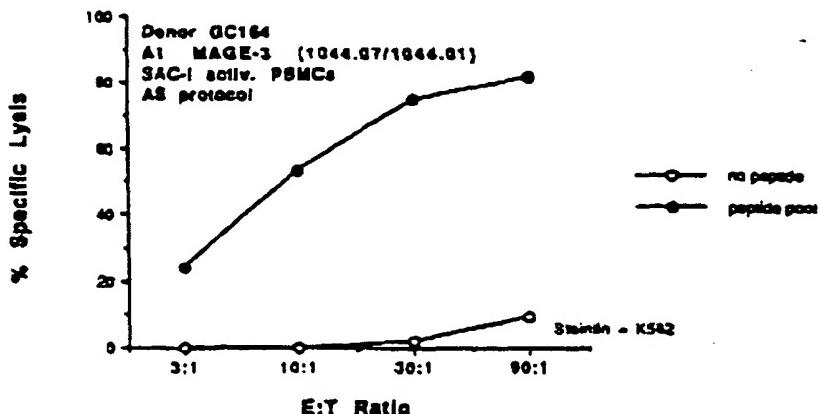
Figure 20

Cytotoxic Activity of an HLA-A1-Restricted CTL Line  
Specific for a MAGE3 Peptide (1044.07)

0035-T603-014553360



**Induction Using Different Methods  
to Load Peptides onto SAC-I Cells.**



00054769 07559960

Figure 22  
Peptide Screening:  
MAGE/A11

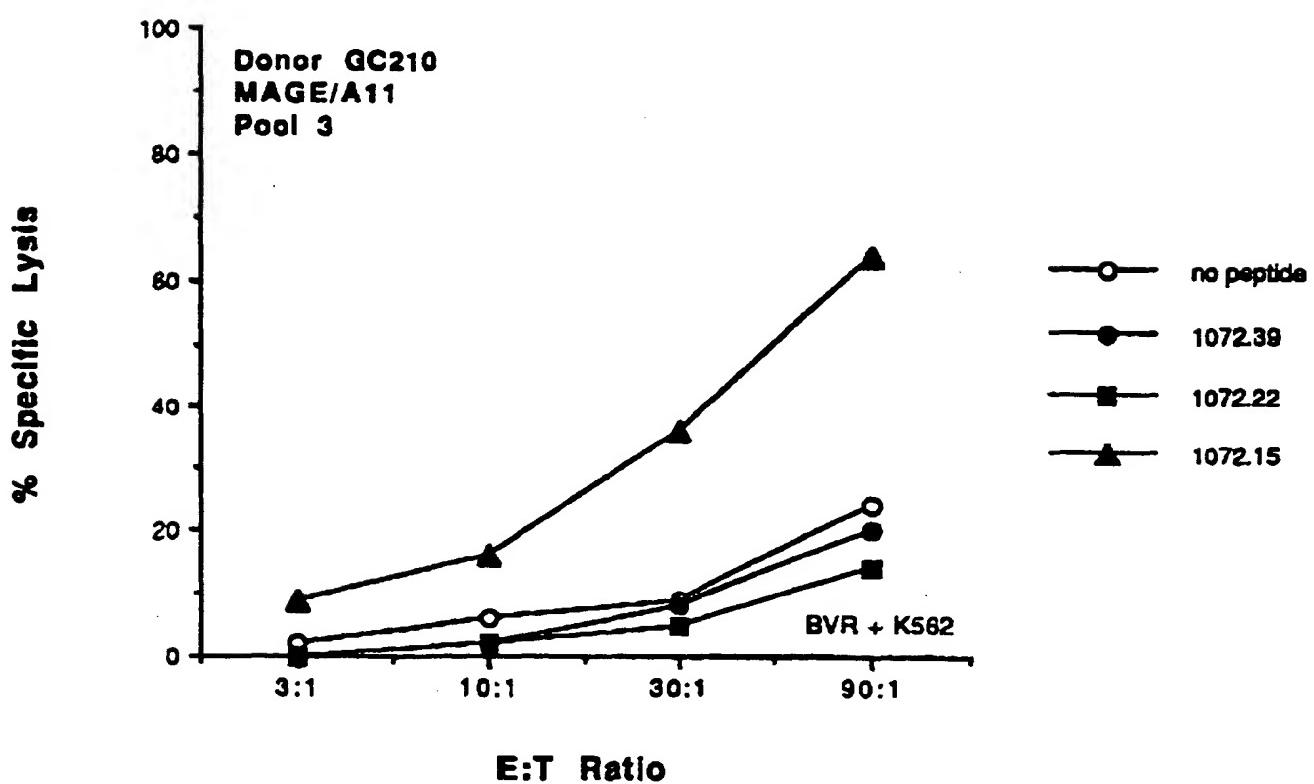
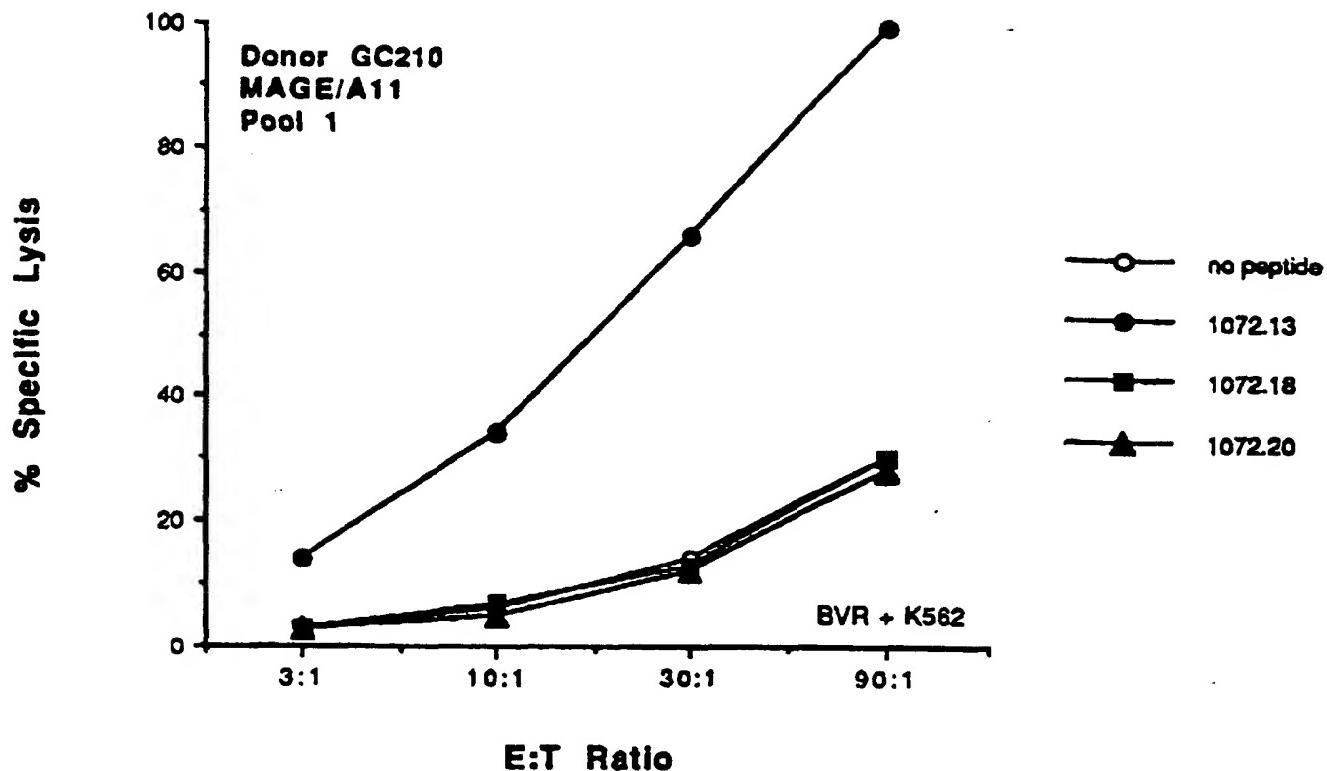


Figure 25  
Peptide Screening:  
HIV/A3

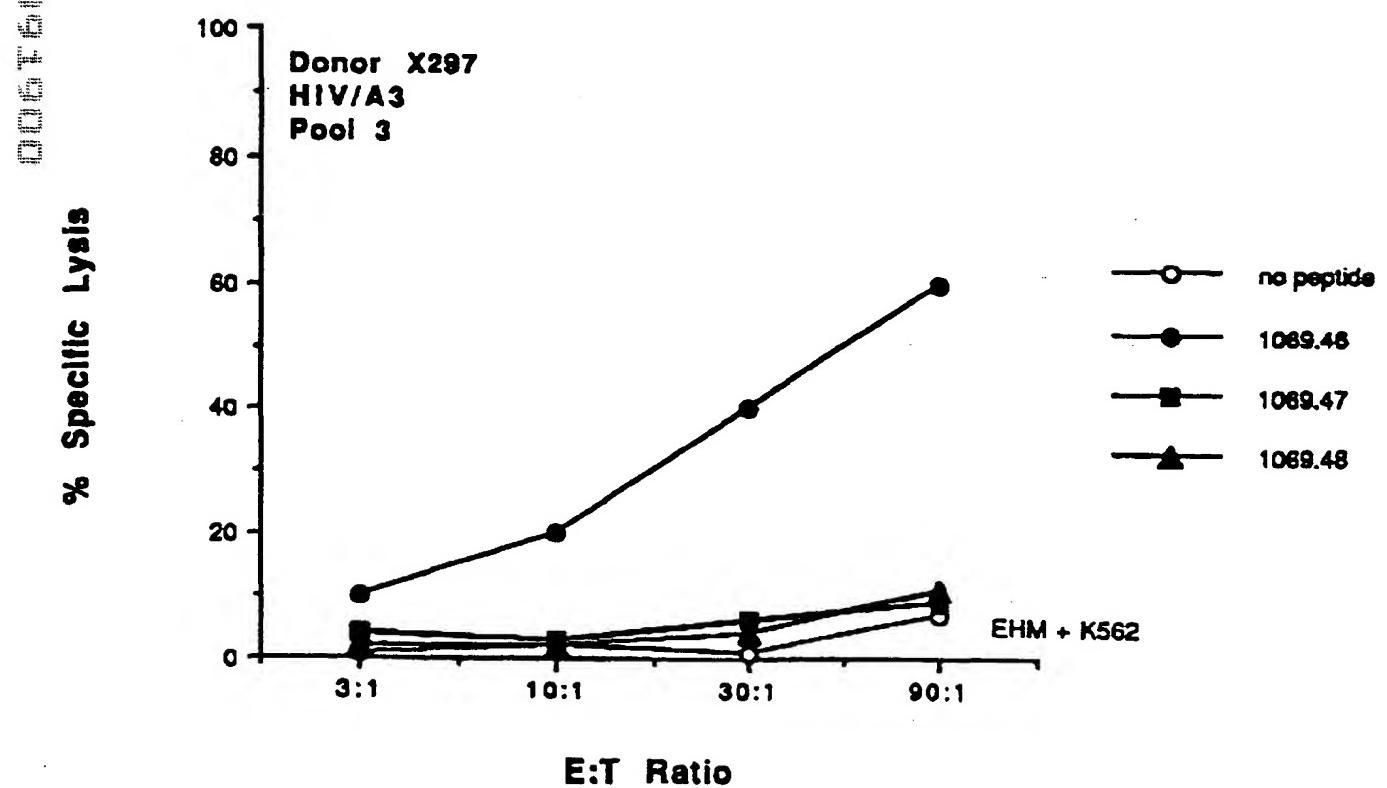
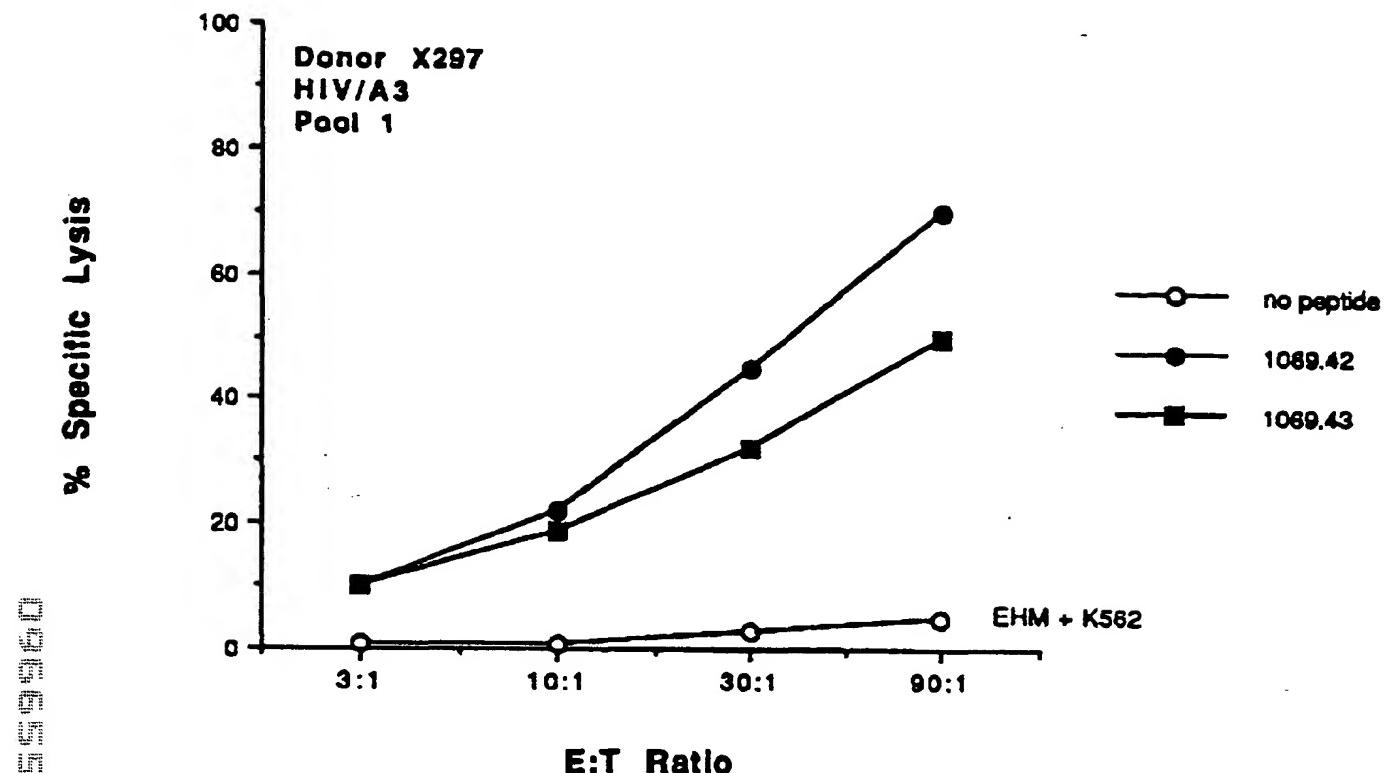


Figure 24

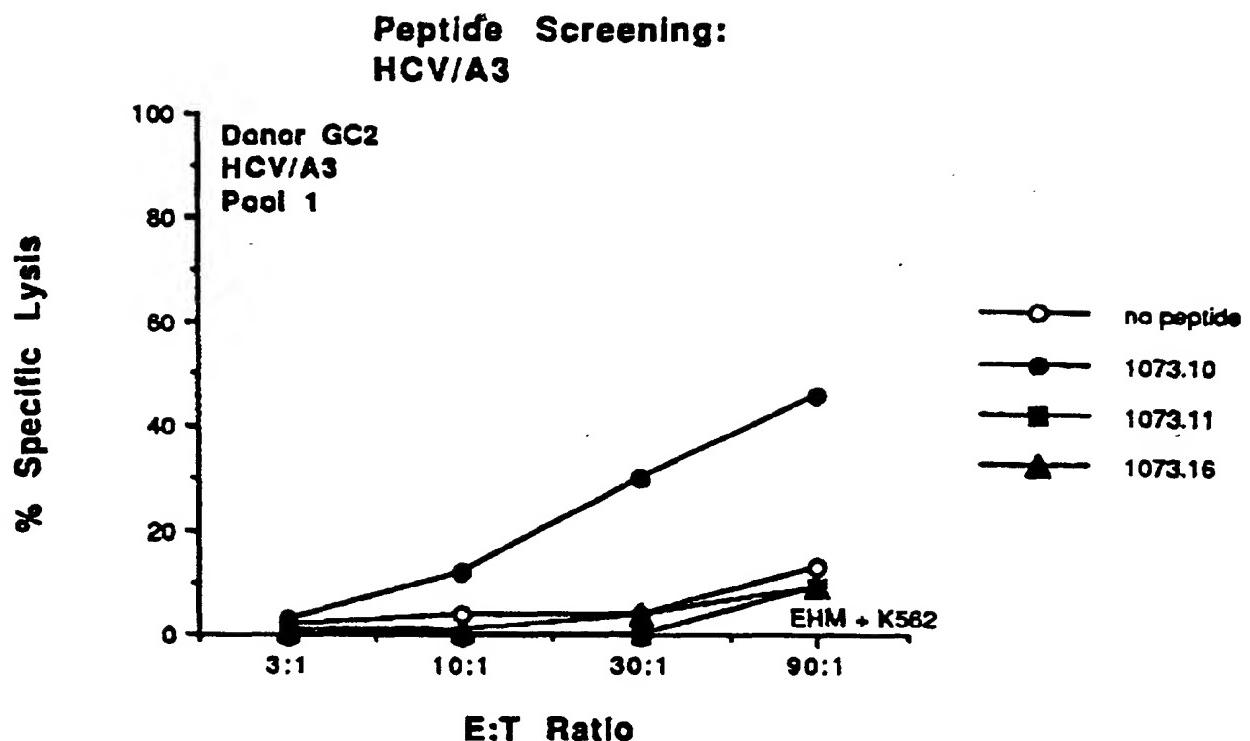


Figure 25

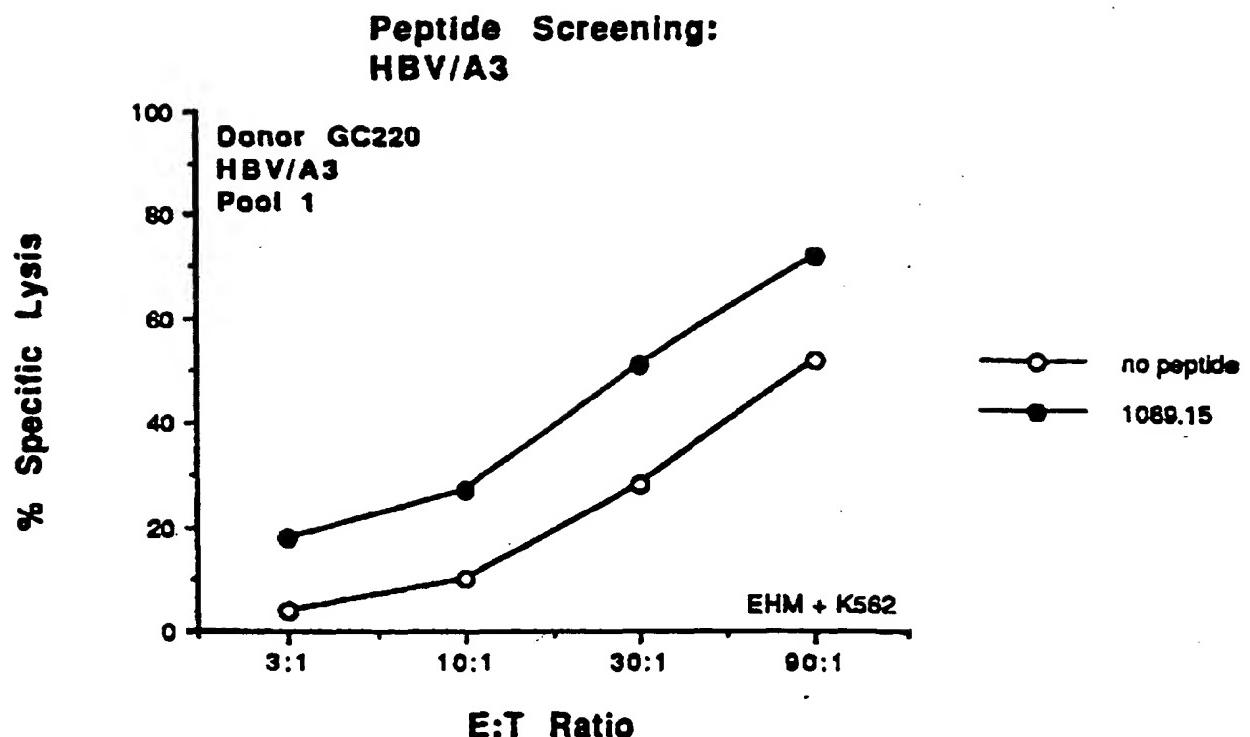


Figure 15

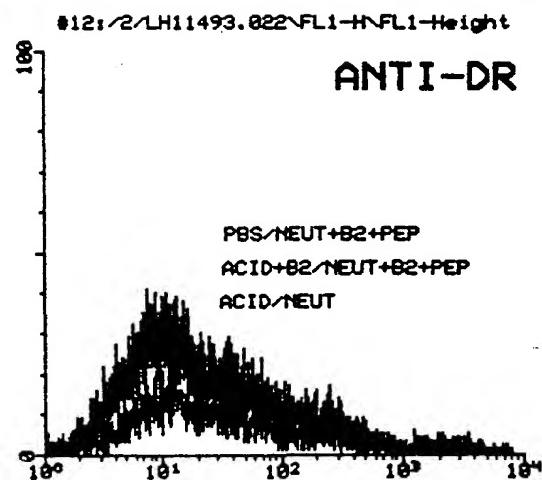
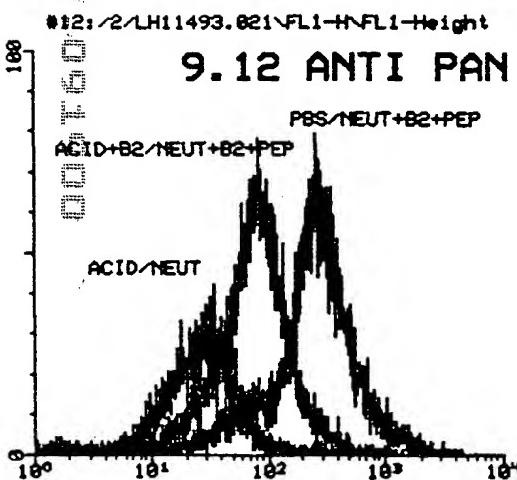
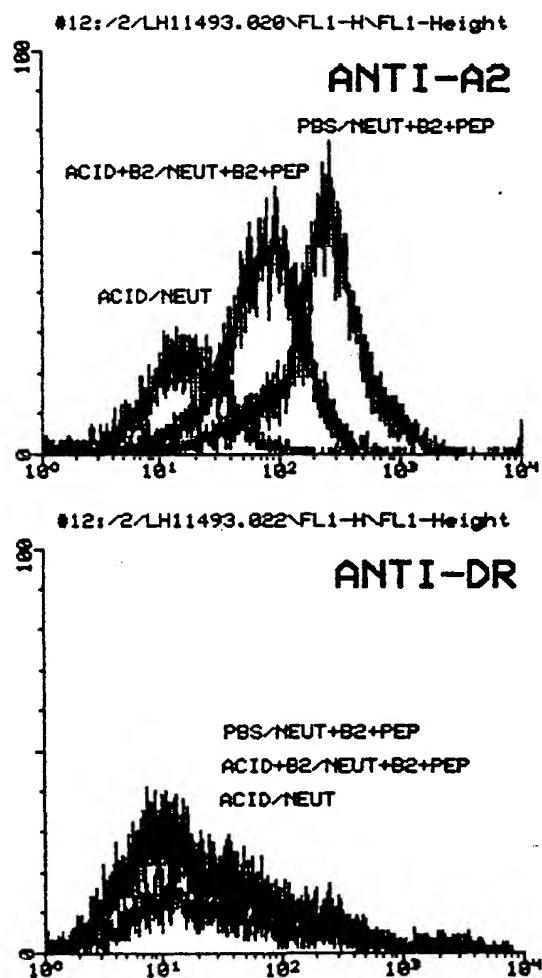
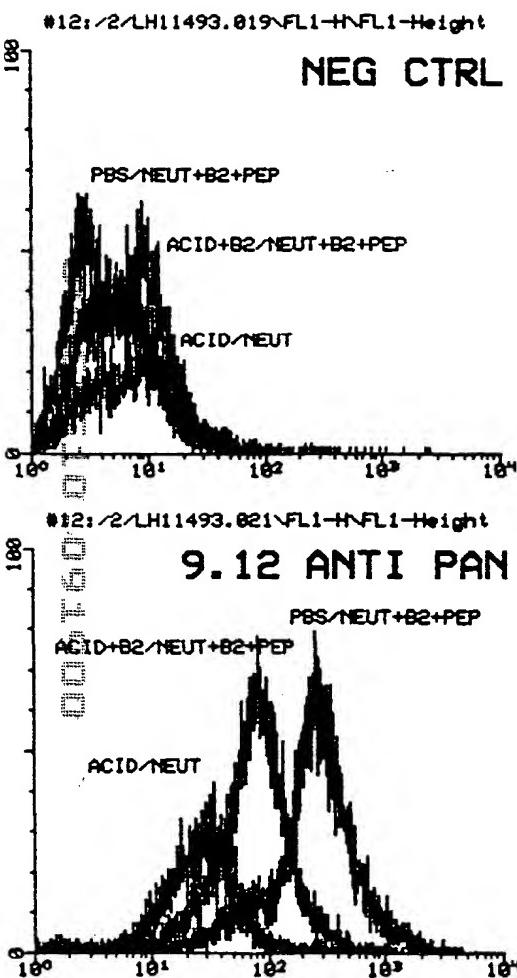


Figure 16

**CTL INDUCTION**  
**GC43 A2.1 CD4 DEPLETED RESPONDER**  
**777.03 + 924.07 + 927.32 POOL**

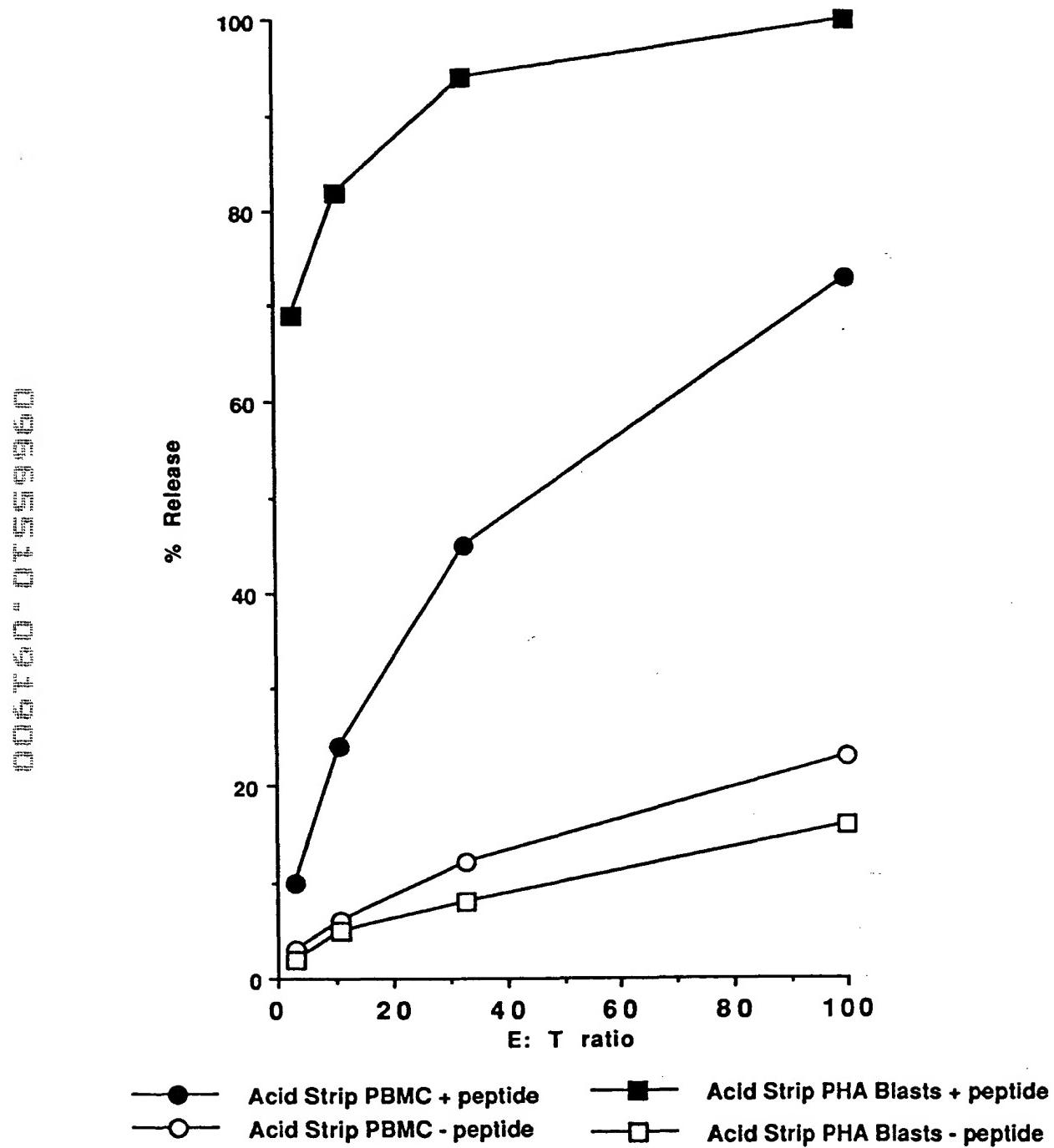


Figure 17



0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100

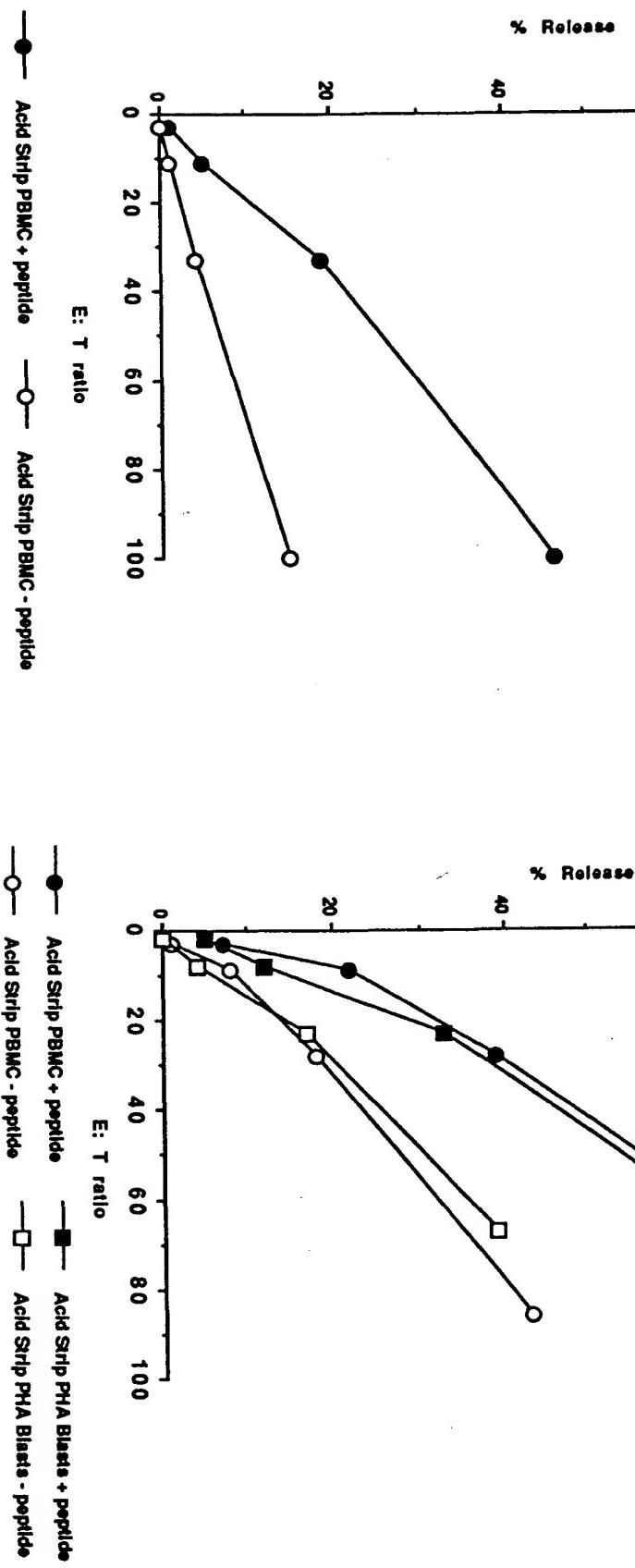


Figure 18

**CTL INDUCTION  
GC49 A2.1 CD4 DEPLETED RESPONDERS  
939.03 PEPTIDE**

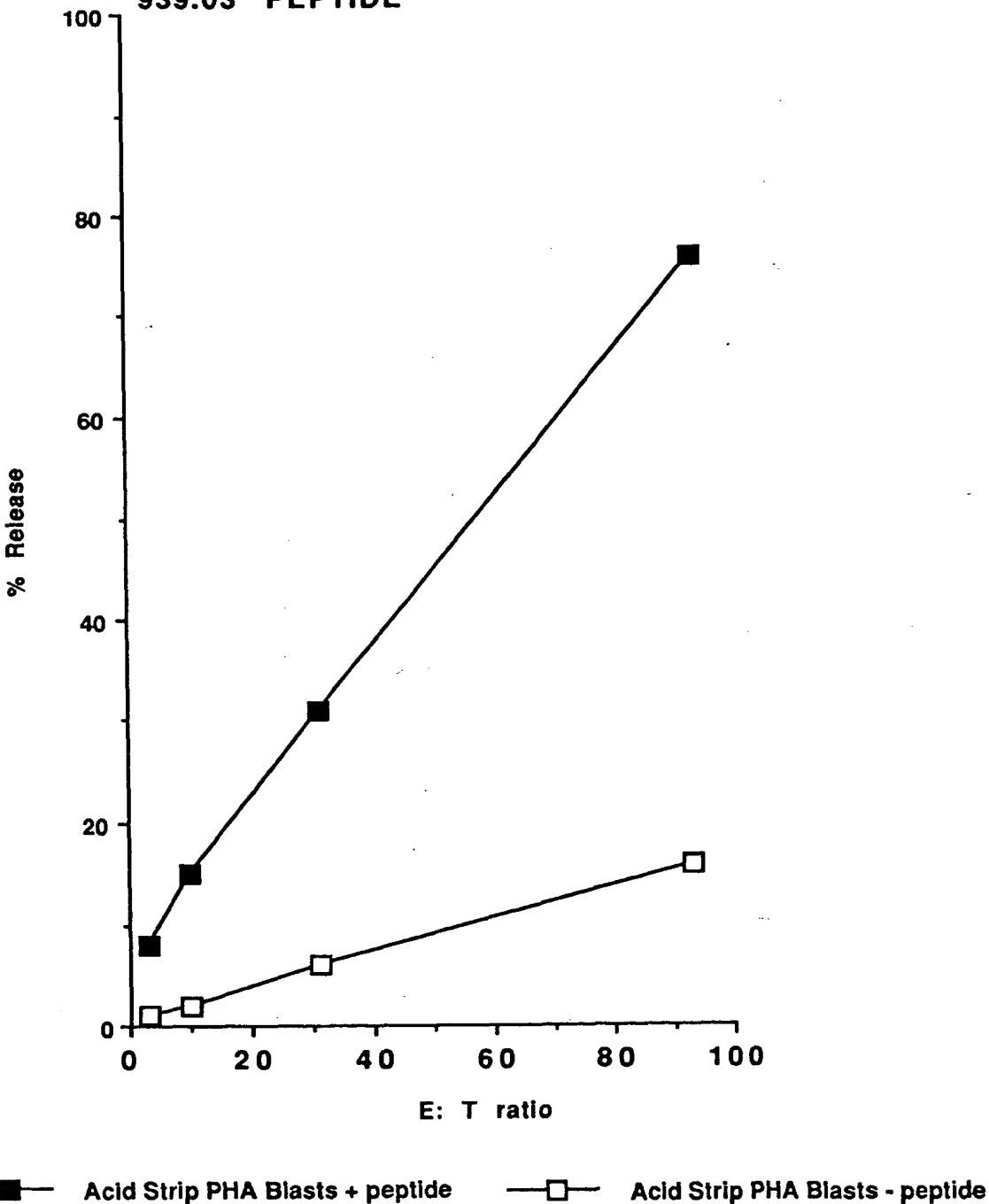
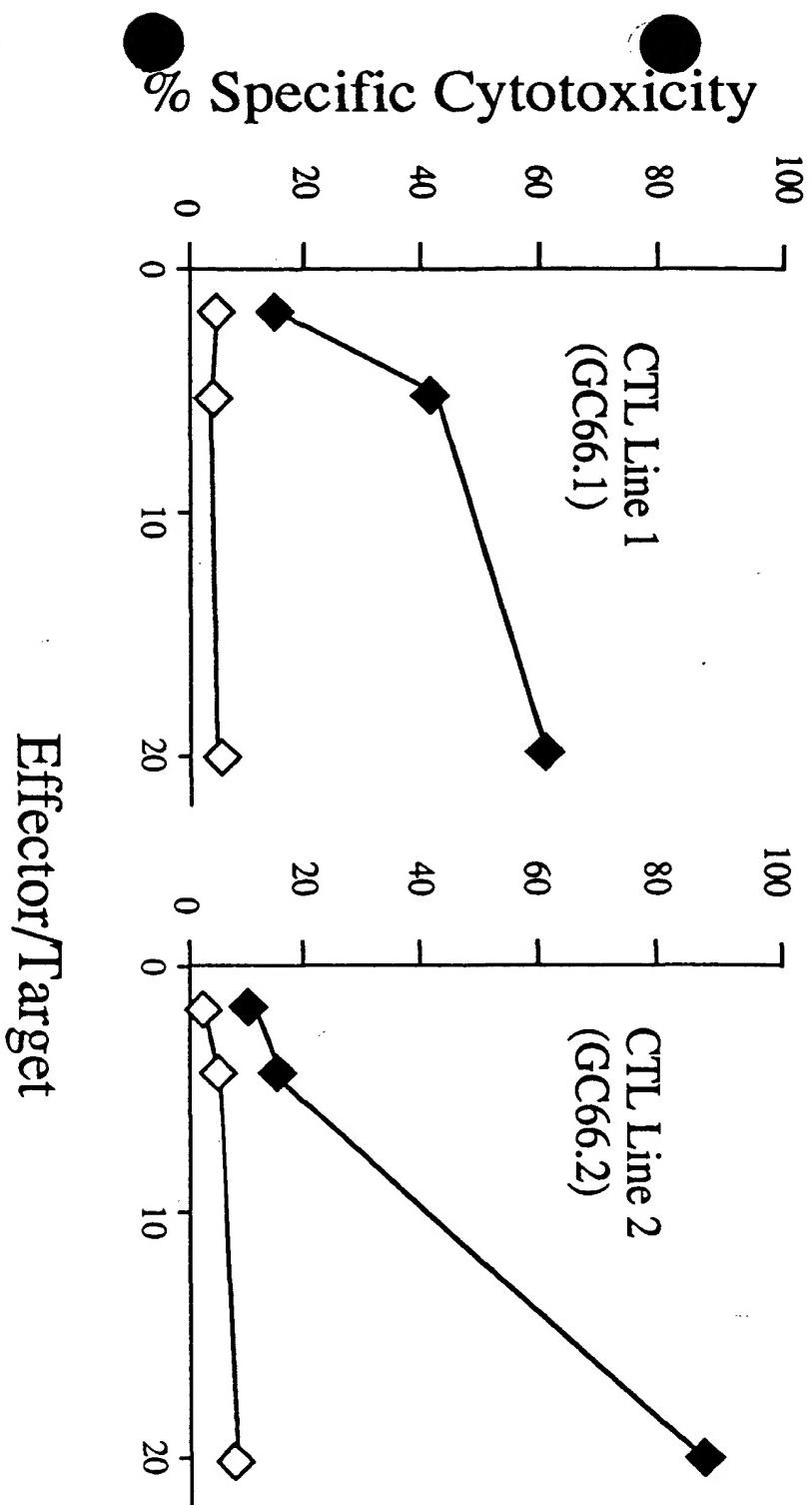


Figure 19

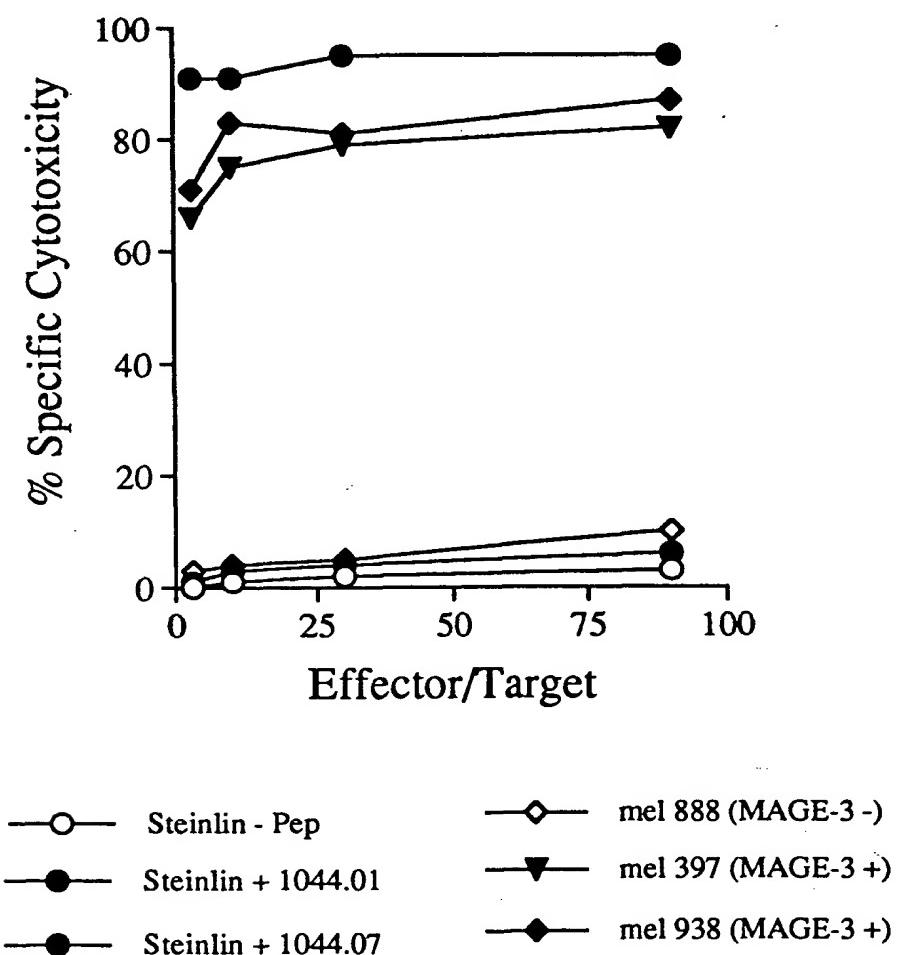
## MAGE1 Peptide Specific, HLA-A1-Restricted CTL Can Kill Melanoma Cells Expressing the Endogenous Antigen



006T60 "IT553950

Figure 20

### Cytotoxic Activity of an HLA-A1-Restricted CTL Line Specific for a MAGE3 Peptide (1044.07)



0005T60 = 014559360

Figure 21  
Induction Using Different Methods  
to Load Peptides onto SAC-I Cells.

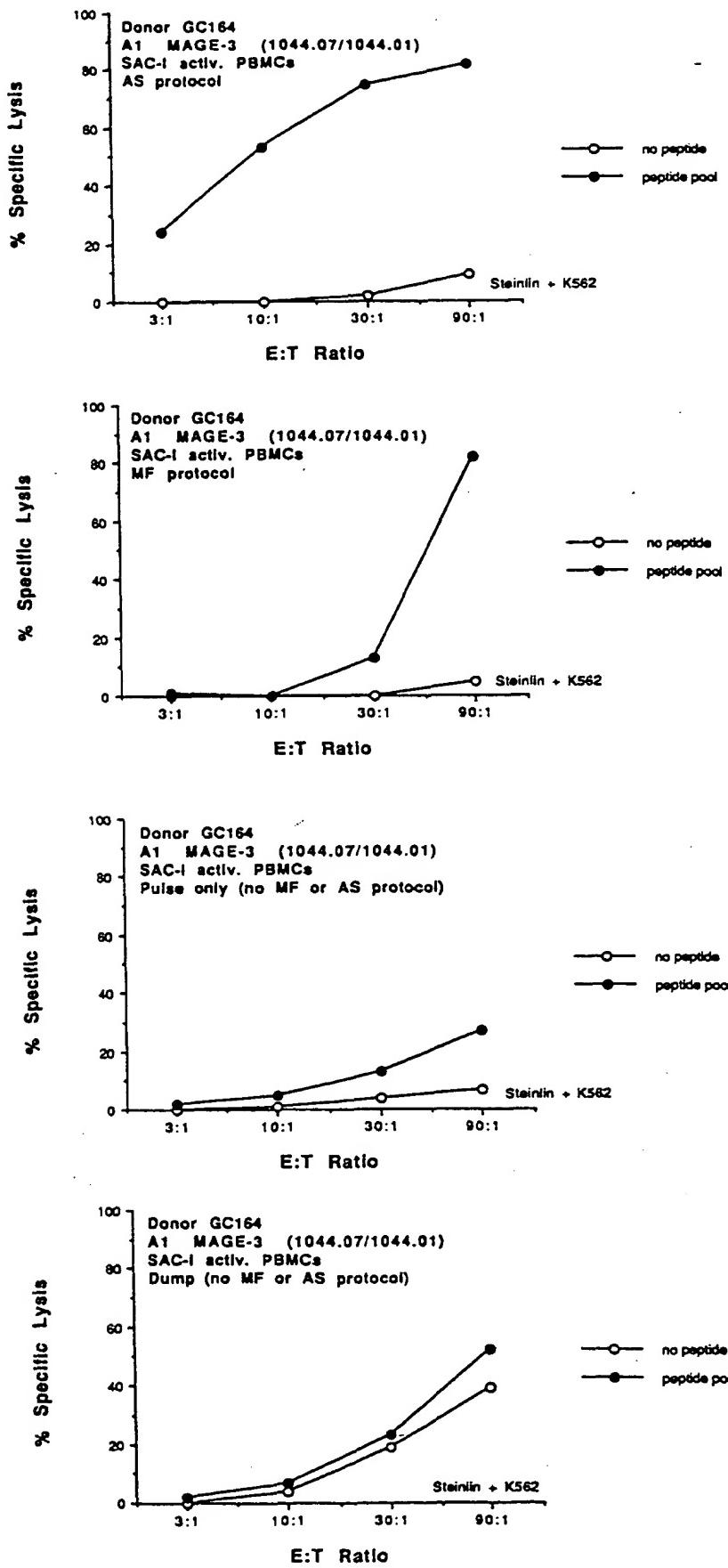


Figure 22  
Peptide Screening:  
MAGE/A11

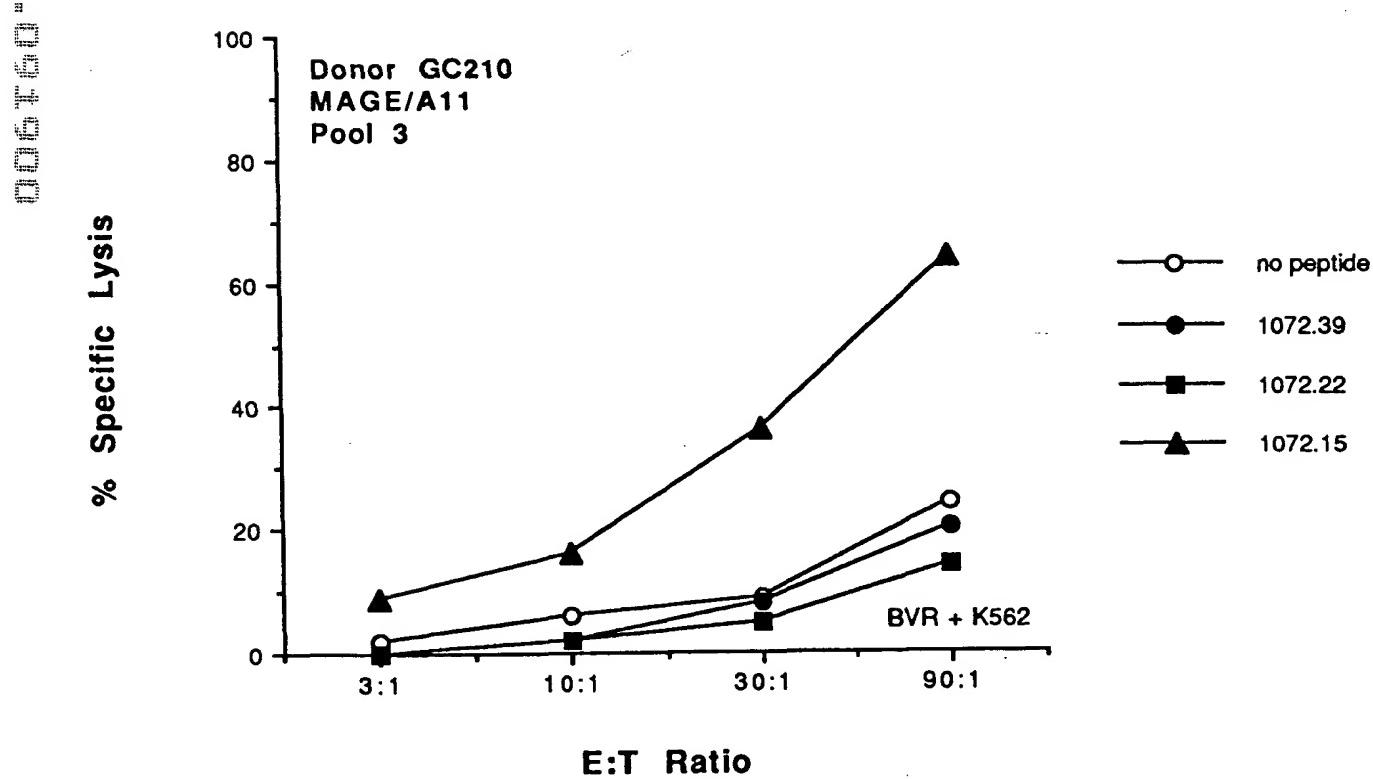
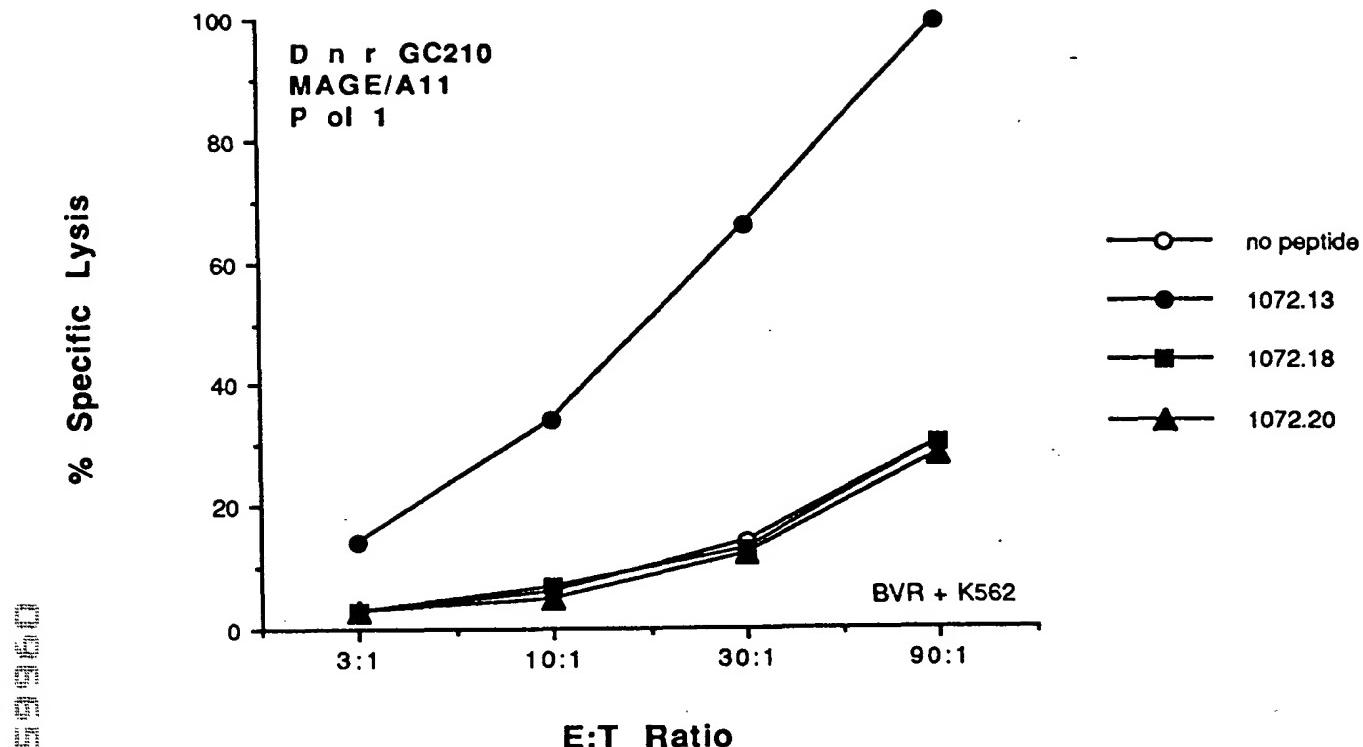


Figure 23  
Peptide Screening:  
HIV/A3

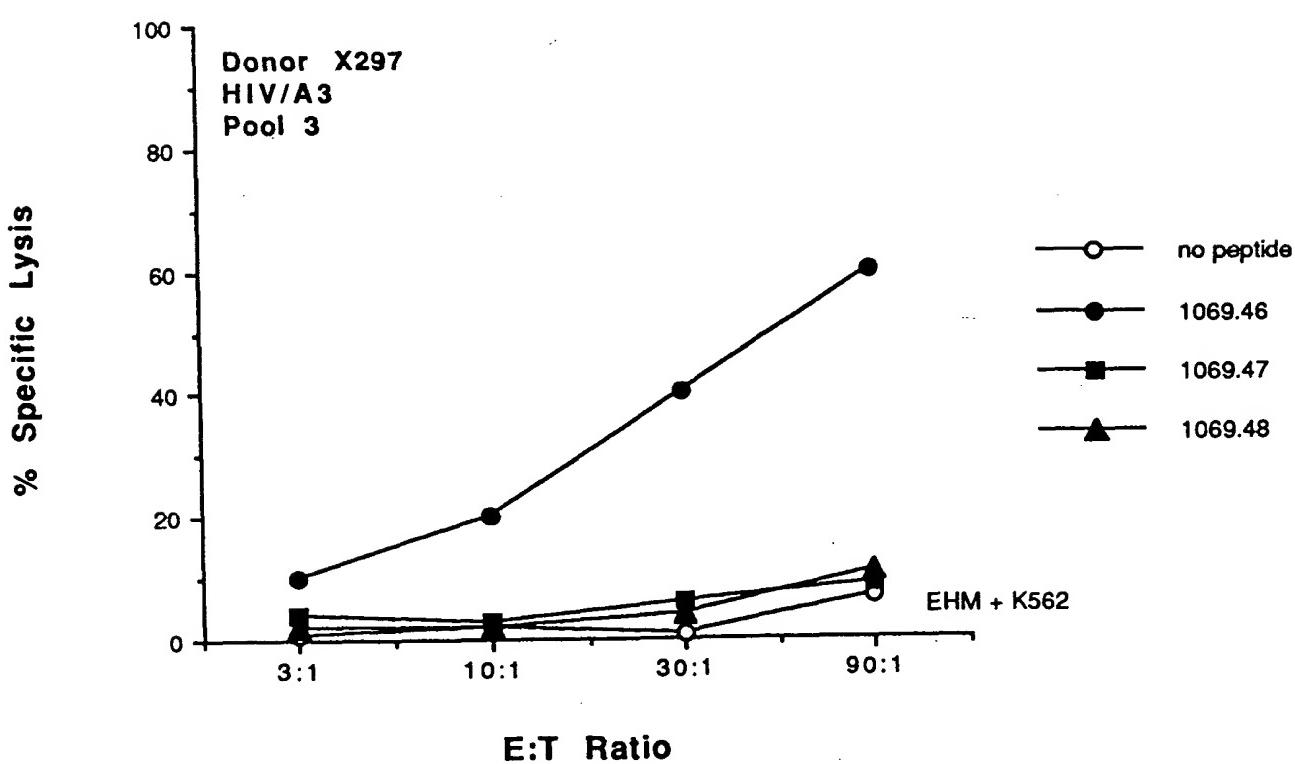
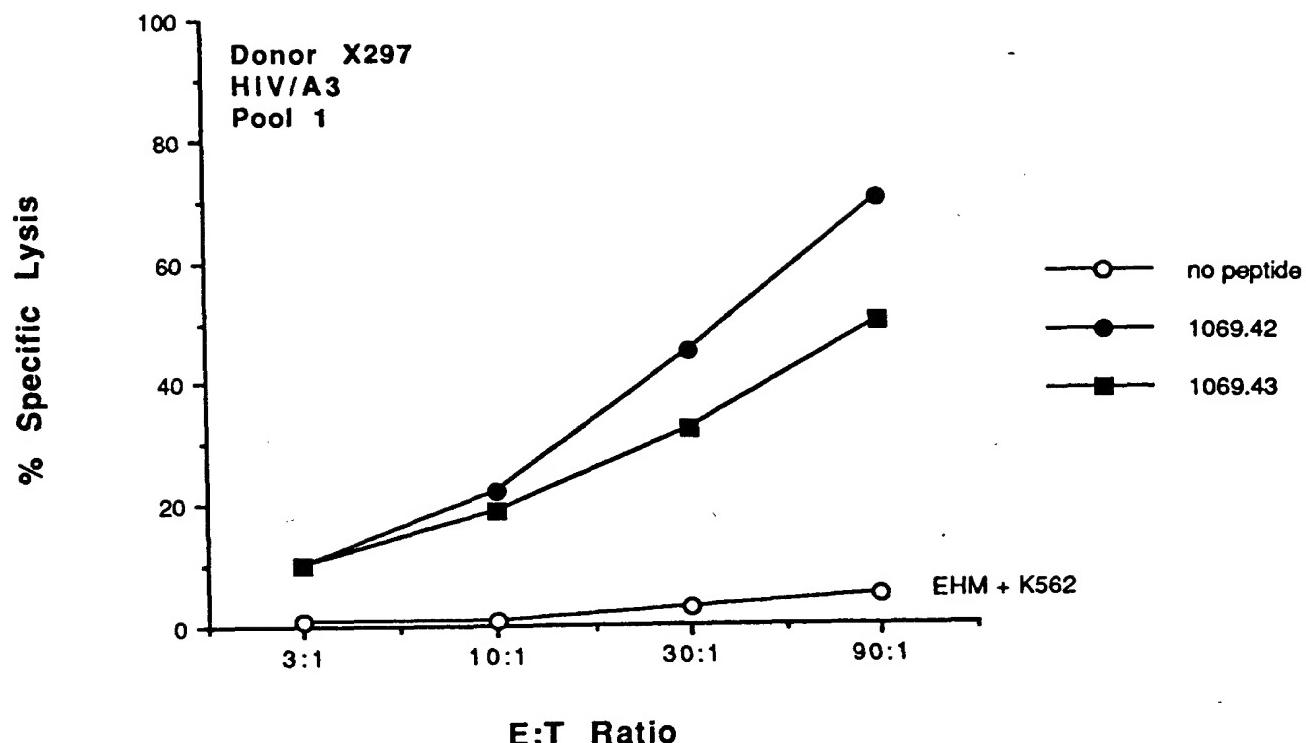


Figure 24

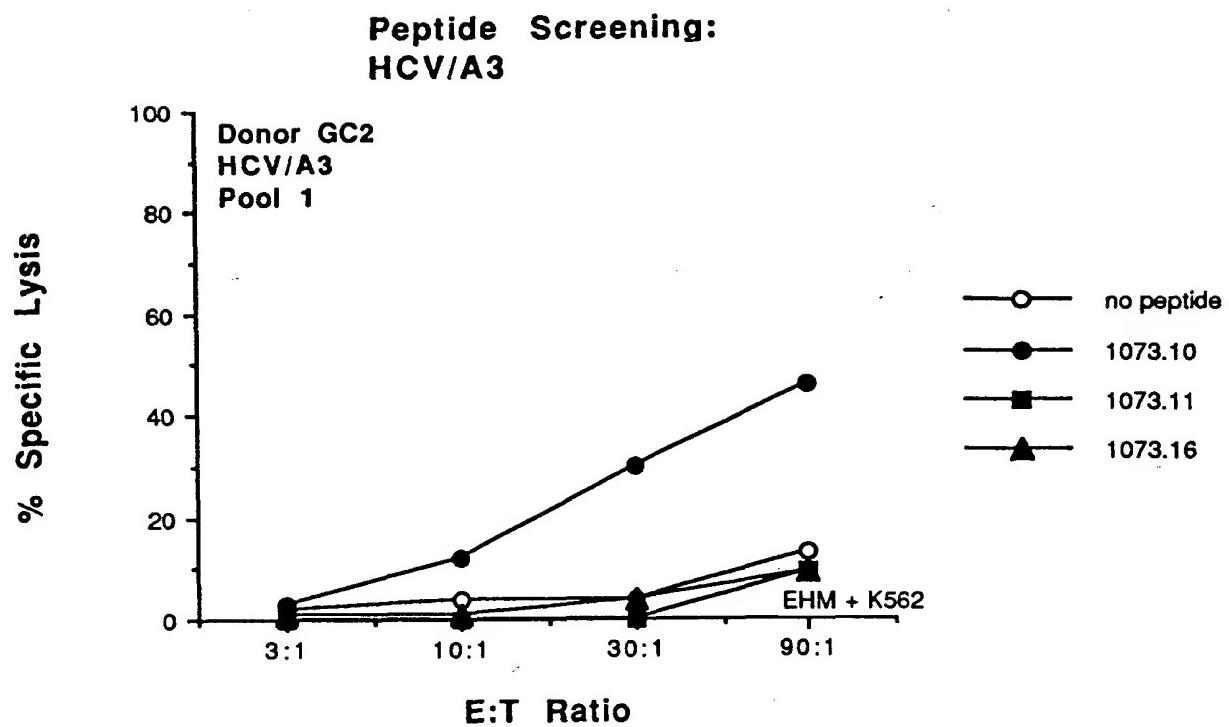


Figure 25

